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Effects of short-term dietary nitrate supplementation on blood pressure, O_2 uptake kinetics, and muscle and cognitive function in older adults

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Kelly J, Fulford J, Vanhatalo A, Blackwell JR, French O, Bailey SJ, Gilchrist M, Winyard PG, Jones AM. Effects of short-term dietary nitrate supplementation on blood pressure, O_2 uptake kinetics, and muscle and cognitive function in older adults. *Am J Physiol Regul Integr Comp Physiol* 304: R73–R83, 2013. First published November 21, 2012; doi:10.1152/ajpregu.00406.2012.—Dietary nitrate (NO_3^-) supplementation has been shown to reduce resting blood pressure and alter the physiological response to exercise in young adults. We investigated whether these effects might also be evident in older adults. In a double-blind, randomized, crossover study, 12 healthy, older (60–70 yr) adults supplemented their diet for 3 days with either nitrate-rich concentrated beetroot juice (BR; 2 × 70 ml/day, ~9.6 mmol/day NO_3^-) or a nitrate-depleted beetroot juice placebo (PL; 2 × 70 ml/day, ~0.01 mmol/day NO_3^-). Before and after the intervention periods, resting blood pressure and plasma [nitrite] were measured, and subjects completed a battery of physiological and cognitive tests. Nitrate supplementation significantly increased plasma [nitrite] and reduced resting systolic (BR: 115 ± 9 vs. PL: 120 ± 6 mmHg; $P < 0.05$) and diastolic (BR: 70 ± 5 vs. PL: 73 ± 5 mmHg; $P < 0.05$) blood pressure. Nitrate supplementation resulted in a speeding of the $\dot{V}O_2$ mean response time (BR: 25 ± 7 vs. PL: 28 ± 7 s; $P < 0.05$) in the transition from standing rest to treadmill walking, although in contrast to our hypothesis, the O_2 cost of exercise remained unchanged. Functional capacity (6-min walk test), the muscle metabolic response to low-intensity exercise, brain metabolite concentrations, and cognitive function were also not altered. Dietary nitrate supplementation reduced resting blood pressure and improved $\dot{V}O_2$ kinetics during treadmill walking in healthy older adults but did not improve walking or cognitive performance. These results may have implications for the enhancement of cardiovascular health in older age.

nitrate; nitrite; nitric oxide; blood pressure; exercise performance; cognitive performance; O_2 uptake kinetics

THE BENEFICIAL EFFECTS OF a vegetable-rich diet upon cardiovascular health (27) and longevity (79) have been well described. These positive effects have been attributed, in part, to inorganic nitrate (NO_3^-), which is particularly rich in leafy greens and beetroot. The NO_3^- anion itself is inert, and any biological effects are likely to be the result of its conversion to the nitrite anion (NO_2^-) in the mouth via facultative anaerobic bacteria on the surface of the tongue (25). When swallowed, NO_2^- can be further converted into nitric oxide (NO) (9), but it is clear that some NO_2^- enters the circulation. The subsequent reduction of NO_2^- to NO and other reactive nitrogen intermediates is facilitated in hypoxia (11). The production of NO via nitric oxide synthase (NOS) is impaired in hypoxia and, thus, it has been proposed that the NO_3^--NO_2^--NO pathway represents a complementary system for NO generation across a wide range of redox states (53). NO is an essential physiological signaling molecule with numerous functions in the body, including the regulation of blood flow, muscle contractility, glucose and calcium homeostasis, and mitochondrial respiration and biogenesis (17, 21, 70).

There is now substantial evidence that dietary NO_3^- supplementation, either in the form of sodium nitrate ($NaNO_3^-$) or beetroot juice, can significantly increase plasma [NO_2^-] and reduce resting blood pressure in young adults (5, 49, 76, 81). Moreover, dietary NO_3^- supplementation may have positive effects upon the physiological response to exercise (5, 50). Supplementation with $NaNO_3^-$ (0.1 mmol·kg^{-1}·day^{-1}; Ref. 50) or beetroot juice (0.5 l/day, containing 5.5 mmol/day of NO_3^-; Ref. 4) resulted in a significant reduction in oxygen uptake ($\dot{V}O_2$) during submaximal cycling. In a recent placebo-controlled study, we reported that beetroot juice supplementation significantly reduced the O_2 cost of treadmill walking and improved exercise tolerance in healthy young adults (47). These results are remarkable because the $\dot{V}O_2$-work rate relationship has traditionally been considered to be independent of age, health status, and aerobic fitness (36). The reduction in the O_2 cost of moderate-intensity exercise following dietary NO_3^- supplementation may be a result of a reduced ATP cost of muscle force production (5) and/or enhanced mitochondrial efficiency (51).

The availability of the NOS substrate L-arginine, and especially the NOS cofactor tetrahydrobiopterin, is lower in older age (23), which together with lower plasma [NO_2^-] (68), a sensitive marker of NOS activity (42), suggests that NO synthesis through the NOS-NO pathway might be impaired with the process of aging. In addition, superoxide (O_2^-) production is increased with aging, which would also be expected to lower NO bioavailability, given the rapid reaction between (O_2^-) and NO to form peroxynitrite (37). Given the positive association between NO and vascular health (34), these aging-related perturbations to NO metabolism might contribute toward the endothelial dysfunction (46, 52) and arterial hypertension (26) that develop with old age. Therefore, it is feasible that dietary NO_3^- supplementation might enhance NO bioavailability and vascular function in older adults.

The ageing process is associated with a number of functional and structural changes to the cardiovascular and muscular systems that may perturb O_2 delivery and utilization. For instance, the ability to increase cardiac output (45) and skeletal

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muscle blood flow (80) during exercise is attenuated with increasing age. Moreover, the distribution of blood flow in the microcirculation, capillary density, and capillary hemodynamics (7, 8, 18, 30, 59, 60, 65), as well as mitochondrial volume density and oxidative function (15, 16) are compromised with aging. There is evidence that $\dot{V}O_2$ kinetics in the transition from a lower to a higher metabolic rate is slowed in older compared with younger adults (3, 14, 22) and that this may be related to a limitation in muscle O_2 delivery (66). The reduction in maximal oxidative phosphorylation capacity in aged muscle (15, 16, 28) might also contribute toward the slower $\dot{V}O_2$ kinetics. Since dietary NO_3^- supplementation has been shown to increase muscle blood flow (19) and the maximal rate of oxidative ATP production (51), it is possible that dietary NO_3^- supplementation might speed $\dot{V}O_2$ kinetics in older adults. Faster $\dot{V}O_2$ kinetics would be expected to reduce metabolic perturbation and fatigue development in the transition from a lower to a higher metabolic rate and may, thus, enhance exercise tolerance. The influence of NO_3^- supplementation on $\dot{V}O_2$ kinetics in older adults has yet to be determined.

Increased NO bioavailability might also enhance brain blood flow and cognitive function in older age. In addition to brain shrinkage in senescence (71), the capacity of the brain to produce ATP via oxidative phosphorylation decreases (10) and, in combination with chronic ischemia of white matter (63), this results in a decline of cognitive function. Furthermore, age-related mitochondrial dysfunction has been associated with the neuronal loss, which is a feature of neurodegenerative diseases (13). Recent studies suggest that NO plays a key role in cerebral vasodilation and blood flow (64), neurotransmission, and the coupling of neural activity to local cerebral blood flow (62). Therefore, dietary NO_3^- supplementation may have the potential to modify cerebrovascular physiology and enhance cognitive function. Indeed, Presley et al. (63) recently reported that dietary nitrate improves regional white matter perfusion in older adults in areas of the brain that are involved in executive functioning and speculated that this may offset the influence of aging on cognitive decline and dementia (32).

The purpose of the present study, therefore, was to assess whether the physiological effects of dietary NO_3^- supplementation reported previously in young adults are also evident in older adults. An additional purpose was to use ^1H magnetic resonance spectroscopy (MRS) brain-scanning techniques to investigate whether NO_3^- supplementation can influence concentrations of key metabolites in the brain, which have been strongly related to cognitive health and whether this translates into improved cognitive function. We hypothesized that dietary supplementation with NO_3^--rich beetroot juice would reduce resting blood pressure, speed $\dot{V}O_2$ kinetics, and lower the O_2 cost of treadmill walking, and improve functional capacity and cognitive function in healthy older adults.

METHODS

Subjects

Twelve older adults (six male and six female) volunteered to participate in this study (mean ± SD; males: age 64 ± 4 yr, height 175 ± 6 cm, body mass 71 ± 9 kg; females: age 63 ± 2 yr, height 163 ± 6 cm, body mass 67 ± 14 kg). All subjects were ostensibly healthy and were not taking medication. None of the subjects was a tobacco smoker or user of dietary supplements. Subjects were screened prior to participation to ensure their suitability for the study. The study was approved by the Institutional Research Ethics Committee. All subjects gave their written, informed consent before the commencement of the study, once the experimental procedures, associated risks, and potential benefits of participation had been described. Subjects were instructed to arrive at the laboratory in a rested and fully hydrated state, at least 3 h postprandial, and to avoid strenuous exercise in the 24 h preceding each testing session. Subjects were also asked to refrain from caffeine and alcohol intake 6 and 24 h before each test, respectively. All tests were performed at approximately the same time of day (±2 h) for each subject.

Procedures

Subjects were required to attend the laboratory on six occasions over a 6-wk period. During *visit 1*, subjects provided a venous blood sample for determination of plasma [NO_2^-], and resting blood pressure (BP) was measured. The subjects then completed a submaximal ramp incremental treadmill exercise test to determine gas exchange threshold (GET). All treadmill tests were performed in a well-ventilated laboratory at 20–22°C on a slat-belt treadmill (PPS-55 Sport, Woodway, Weil am Rhein, Germany) set at a 1% gradient (35). Initially, subjects completed 3 min of baseline walking exercise at 1 km/h, after which the belt speed was increased by 1 km/h every minute. Subjects were instructed to exercise until they were breathing heavily, the exercise became challenging, or that the treadmill speed was uncomfortably fast for them to continue. Alternatively, if the subject's heart rate (HR) reached a predetermined value (80% of age-predicted maximum), the exercise test was terminated. The breath-by-breath pulmonary gas-exchange data were collected continuously during the incremental test and averaged over consecutive 10-s periods. The GET was determined from a cluster of measurements, including *1*) the first disproportionate increase in CO_2 production ($\dot{V}CO_2$) from visual inspection of individual plots of $\dot{V}CO_2$ vs. $\dot{V}O_2$; *2*) an increase in expired ventilation ($\dot{V}E$)/$\dot{V}O_2$ with no increase in $\dot{V}E/\dot{V}CO_2$; and *3*) an increase in end-tidal O_2 tension with no fall in end-tidal CO_2 tension. Subsequently, treadmill speeds that would require 80% of the GET (moderate-intensity exercise) were calculated, with account taken of the mean response time for $\dot{V}O_2$ during ramp exercise (i.e., two-thirds of the ramp rate was deducted from the treadmill speed at GET). During *visit 1*, subjects were also given a cognitive training session to familiarize them with the process, format, and required responses to all computer-based cognitive tests that were to be utilized during the study. Following this, subjects were assigned in a double-blind, randomized, crossover design to consume 140 ml/day of NO_3^--rich BR or NO_3^--depleted beetroot juice (PL) for 2.5 days prior to each of their subsequent laboratory visits. The subjects were instructed to follow their normal dietary habits throughout the experimental period and asked to record and replicate their diet as closely as possible between conditions during each of the 2.5-day supplementation period. Subjects were also requested to abstain from using antibacterial mouthwash and chewing gum throughout the study since this can markedly reduce the concentration of oral bacteria responsible for the reduction of NO_3^- to NO_2^- (29).

During *visits 2* and *3,* venous blood samples were drawn, and resting BP was measured. The subjects were then asked to complete step-transition, walking exercise tests on a treadmill for the determination of pulmonary $\dot{V}O_2$ dynamics. The protocol involved two 6-min bouts of moderate-intensity walking (at 80% GET). Each exercise bout involved an abrupt transition to the target speed initiated from a slow walking baseline (1 km/h), with the two exercise bouts separated by 10 min of passive recovery. Following the step-exercise tests, 10 min of passive recovery was taken before the completion of a 6-min walk test (6MWT) to assess functional capacity. The 6MWT was completed following the appropriate guidelines and standardizations, as suggested in the American Thoracic Society Statement: Guidelines

for the 6MWT (2) with total distance covered being recorded. The test was completed on a straight, flat track. Both the subject and the researcher were blind as to which supplement was being tested, and any encouragement during the test was standardized. HR was recorded throughout both the treadmill step-exercise tests and the 6MWT. After a further 10-min passive recovery, subjects were asked to complete a number of computer-based cognitive function tests, which assessed the impact of the supplementation on the speed and accuracy of cognitively demanding tasks. There were three cognitive tests in total.

Serial subtractions. The original verbal Serial Sevens subtraction test has been employed in a number of other studies and is included as part of the Mini-Mental State Examination for dementia screening. The current study utilized a modified, 4-min, computerized version of the serial subtraction task (67), which was made up of 2 min of serial 3s followed by 2 min of serial 7s subtractions. Before each 2-min section, a standard instruction screen requested the subject to count backward in 3s or 7s, as quickly and as accurately as possible, using the keyboard's linear number keys to enter each response. The instructions also made it clear to subjects that if they were to make a mistake they should carry on subtracting from the new incorrect number. A random starting number between 800 and 999 was presented on the computer screen, which was cleared by the entry of the first response. Subsequently each three-digit response was represented on the screen by three asterisks. Performance data (total number of subtractions and number of errors) were calculated for the serial 3s and 7s responses separately. In the case of an incorrect response, subsequent responses were scored as positive if they were correct in relation to the new number.

Rapid visual information processing. This task has been used previously to study the cognitive effects of psychotropic drugs. The subject was asked to monitor a continuous series of single digits to identify targets of three consecutive odd or three consecutive even digits. The digits were presented on the computer screen at a rate of 100/min in pseudo-random order, and the participant was required to respond to the detection of a target string by pressing the space bar as quickly as possible. The task was continuous and lasted 5 min in total, with 8 correct target strings being presented per minute. The subjects were scored for the number of target strings correctly detected, average reaction time (ms) for correct detections, and number of false alarms.

Number recall. The subjects were initially presented with a three-digit number on the screen and given 3 s to learn the number. The number was then removed, and subjects were prompted to recall the number verbally to the researcher in either forward or backward form. After 12 three-digit numbers, the subject was presented with 12 four-digit numbers, then 12 five-digit numbers, and so on. The time given to subjects to learn the number increased in a linear fashion, on the order of one additional second per one additional number. The task was terminated when the subject gave three consecutive incorrect backward responses and three consecutive incorrect forward responses. Subjects were scored for number of correct forward responses, number of correct backward responses and given a combined total.

Visit 4 was performed with no supplementation and acted as a familiarization session for subjects to the exercise protocols that were to be performed in *visits 5* and *6*.

During *visits 5* and *6,* subjects were required to complete a single-leg, knee-extension exercise test while lying prone in the bore of a 1.5 T superconducting MR scanner (Philips Gyroscan Clinical Intera). Subjects were familiarized with the dimensions of the scanner and the knee-extension exercise in a purpose-built mock scanner during *visit 4*. The exercise protocol consisted of unilateral knee extensions with the right leg using a custom-built nonferrous ergometer. The foot was fastened securely with Velcro straps to a padded foot brace, which was connected to the ergometer load basket via a simple rope and pulley system. Knee extensions over ~0.22 m were completed at a constant frequency, set in unison with the magnetic pulse sequence (40 pulses/min), to ensure the quadriceps muscles were positioned in the same phase of contraction during each MR pulse acquisition. The subjects were visually and audibly cued via a display consisting of two vertical bars, one that moved at a constant frequency of 0.67 Hz and one that monitored foot movement via a sensor in the ergometer pulley system. Because we used a pulse-acquire sequence during the exercise protocol that was pulse acquired, the signal originates from the muscle and is, therefore, relatively insensitive to a subject's movement. Even so, to prevent displacement of the quadriceps relative to the MRS coil during the exercise, Velcro straps were fastened over the subject's legs, hips, and lower back. Following an initial 2-min rest period, subjects performed a 4-min low-intensity exercise bout to assess the muscle metabolic response. This bout was repeated after a 6-min rest period. A further 4-min rest period was followed by two bouts of high-intensity exercise of 24-s duration, which were separated by a 4-min rest period. The intensity of these 24-s exercise bouts was carefully selected to ensure a significant depletion of muscle [PCr] without a significant reduction of pH relative to baseline values. Following the exercise, participants were asked to lie still in a supine position in the bore of the scanner for ~45 min, with their head comfortably secured within an 8-channel SENSE head coil. ^{1}H MRS brain measurements of *N*-acetyl aspartate (NAA), creatine (Cr), choline (Ch), myo-Inositol (mI) concentrations and apparent diffusion coefficients (ADC) of both white and gray matter were recorded.

Supplementation Protocol

After completion of the nonsupplemented *visit 1,* subjects were assigned in a double-blind, randomized, crossover design to receive 2.5 days of dietary supplementation prior to *visits 2, 3, 5,* and *6*. The supplements were either concentrated NO_3^--rich BR (2 × 70 ml/day, organic beetroot juice, each containing ~4.8 mmol NO_3^-, Beet it, James White Drinks, Ipswich, UK) or NO_3^- -depleted PL (2 × 70 ml/day, organic beetroot juice containing ~0.01 mmol NO_3^-, Beet it, James White Drinks, Ipswich, UK). The PL beverage was created by passage of the juice, before pasteurization, through a column containing Purolite A520E ion exchange resin, which selectively removes NO_3^- ions (47). The PL was similar to the BR in appearance, taste, and smell. Subjects were instructed to consume one of the 70-ml beverages in the morning and the other in the afternoon of *day 1* and *2* of supplementation, and then in the morning and 2.5 h prior to their visit on *day 3* of supplementation. At least 72-h washout period separated each supplementation period, and subjects were instructed to maintain their normal daily activities and food intake throughout the study. Subjects were warned that supplementation may cause beeturia (red urine) and red stools temporarily but that this side effect was harmless.

Measurements

Prior to each testing session, blood pressure of the brachial artery was measured using an automated sphygmomanometer (Dinamap Pro, GE Medical Systems, Tampa, FL). Subjects were seated at rest for 10 min prior to the measurements. A total of four measurements were recorded, with the mean of the final three measurements being calculated. Mean arterial pressure (MAP) was calculated as 1/3 × systolic pressure +2/3 × diastolic pressure. The mean of the systolic, diastolic, and MAP measurements made in the two BR- and PL-supplemented sessions (treadmill walking exercise session and MR scanner session) was calculated.

Plasma [NO_2^-] was used as a biomarker for NO availability (42, 52). To obtain plasma [NO_2^-], venous blood samples (~4 ml) were drawn into lithium-heparin tubes (Vacutainer, Becton Dickinson, Franklin Lakes, NJ). Within 3 min of collection, samples were centrifuged at 4,000 rpm and 4°C for 10 min. Plasma was extracted and immediately frozen at −80°C for later analysis of [NO_2^-]. Prior to, and regularly during analysis, all glassware, utensils, and surfaces

were rinsed with deionized water to remove any residual NO_2^-. After plasma samples were thawed at room temperature, they were initially deproteinized using cold ethanol precipitation. The ethanol was chilled to 0°C, and then 0.4 ml of cooled ethanol was combined with 0.2 ml of plasma. Samples were then vortexed and centrifuged at 14,000 rpm for 5 min, with the supernatant being removed. The [NO_2^-] of the deproteinized plasma samples was determined using a modification of the chemiluminescence technique (4).

During all laboratory exercise tests, pulmonary gas exchange and ventilation were measured continuously with subjects wearing a nose clip and breathing through a mouthpiece and impeller turbine assembly (Jaeger Triple V). The inspired and expired gas volume and gas concentration signals were continuously sampled at 100 Hz, the latter using paramagnetic (O_2) and infrared (CO_2) analyzers (Oxycon Pro, Jaeger, Hoechberg, Germany) via a capillary line connected to the mouthpiece. The gas analyzers were calibrated before each test with gases of known concentration, and the turbine volume transducer was calibrated using a three-liter syringe (Hans Rudolph, Kansas City, MO). Pulmonary gas exchange variables were calculated and displayed breath-by-breath. HR was measured using short-range radiotelemetry (model 610; Polar Electro Oy, Kempele, Finland).

During the MRS exercise measurements, subjects lay in the prone position, inside a whole body scanner. A 6-cm ^{31}P transmit/receive surface coil was placed within the subject bed in a way that it was centered over the quadriceps muscle of the right leg. Initially, fast-field echo images were acquired to determine whether the muscle was correctly positioned in relation to the coil. This was aided by the placement of cod liver oil capsules (yielding high-intensity signal points within the image) adjacent to the coil, enabling its orientation relative to the muscle volume under examination to be assessed. A number of preacquisition procedures were performed to optimize the signal from the muscle. Tuning and matching of the coil were carried out, enabling maximal energy transfer between the coil and muscle. An automatic shimming protocol was undertaken within a volume that defines the quadriceps, enhancing homogeneity of the local magnetic field. Throughout all exercise and rest periods, data were acquired every 1.5 s with a spectral width of 1,500 Hz and 1,000 data points. Phase cycling with four phase cycles was employed, which led to the acquisition of a spectrum every 6 s. The resulting spectra were quantified via peak fitting, assuming prior knowledge, using the jMRUI (version 3) software package (61) employing the Advanced Method for Accurate, Robust, and Efficient Spectra (AMARES) fitting algorithm (75). Spectra were fitted assuming the presence of the following peaks: P_i, phosphodiester, PCr, α-ATP (2 peaks, amplitude ratio 1:1), γ-ATP (2 peaks, amplitude ratio 1:1), and β-ATP (3-peaks, amplitude ratio 1:2:1).

Absolute metabolite values were established via a technique similar to that described previously (40). Prior to the exercise protocols, spatially localized spectroscopy was undertaken to determine the relative signal intensities obtained from a phosphoric acid source and P_i from the subject's right quadriceps. A subsequent unsaturated scan was used to compare the signals obtained from the phosphoric acid standard with an external P_i solution, where the localized volume sampled within the muscle was the same dimensions and distance from the coil as the P_i solution. This allowed the calculation of muscle P_i concentration, following corrections for relative coil loading. Subsequently, absolute values of [PCr] and ATP concentrations were calculated via the ratio of P_i to PCr and P_i to ATP. Intracellular pH was calculated using the chemical shift of the P_i spectral peak relative to the PCr peak (72). In addition to this, ADP concentration ([ADP]) was calculated as described previously (39).

^{1}H MRS was performed using an eight-channel SENSE head coil in the left frontal white matter and the occipito-parietal gray matter in single voxels of 2 × 2 × 2 cm. Following automated shimming and pulse angle determination, a point-resolved spectroscopy (PRESS) sequence was undertaken with an echo time of 33 ms and a repetition time (TR) of 2,000 ms with 512 samples acquired and a bandwidth of 1,000 Hz. In each region, the sequence was repeated twice, once with, and once without, water suppression. For the water suppression sequence, an excitation prepulse was applied at the water frequency with an 80-Hz window, prior to the PRESS sequence, which consisted of 128 repetitions averaged together with 16 phase cycles. For the nonwater-suppressed sequence, no prepulse was applied and 32 repetitions were averaged with 16 phase cycles. Quantification was undertaken in jMRUI (ver. 3) employing the AMARES fitting algorithm (75). For the water-suppressed sequence, the residual water peak was removed via an Hankel Lanczos Singular Values Decomposition filter prior to peak fitting, from which the areas of the NAA, Cr, Ch, and mI peaks were calculated. Subsequently, once a correction had been made for the relative number of averages employed in the water-suppressed and nonwater-suppressed sequences, ratios of NAA:water, Ch:water, Cr:water, mI:water, NAA:Ch, NAA:Cr and NAA:(Cr + Ch) were calculated. In addition to this, diffusion images were acquired using an eight channel SENSE head coil with a single-shot echo-planar imaging sequence with 15 directions and b values of 0 and 800 s/mm^{-2}. Images were acquired at an axial-oblique orientation with a TR of 11,000 ms, an echo time of 66 ms, an in-plane resolution of 2 × 2 mm, and a slice thickness of 2 mm. Regions of interest were selected in the anterior cingulated gyrus, the dorsolateral prefrontal cortex, and the subcortical white matter of the frontal lobe, and ADC were calculated using the $b = 0$ and isotopic $b = 800$ s/mm^{-2} images, such that ADC $= -(1/800)$ ln (S/S_0), where S is the signal intensity in the selected ROI for the $b = 800$ s/mm^{-2}, and S_0 is the image intensity for the corresponding $b = 0$ image.

Data Analysis

Oxygen uptake. The breath-by breath $\dot{V}O_2$ data from each test were initially examined to exclude errant breaths caused by coughing and swallowing, with those values lying more than four SDs from the local mean being removed. The breath-by-breath data were subsequently linearly interpolated to provide second-by-second values, and, for each individual, identical repetitions were time-aligned to the start of exercise and ensemble-averaged. This approach enhances the signal-to-noise ratio and improves confidence in the parameters derived from the modeling process (82). A nonlinear least-squares algorithm was used to fit the data. With only two transitions and a relatively low-response amplitude, however, we elected to describe the overall $\dot{V}O_2$ kinetics using the mean response time (MRT), which was calculated by fitting a single exponential curve to the data with no time delay from the onset to the end of exercise. An iterative process was used to minimize the sum of the squared errors between the fitted function and the observed values. We then calculated the oxygen deficit (O_2df) as the product of the $\dot{V}O_2$ response amplitude (baseline to exercise steady-state) and the MRT. $\dot{V}O_{2baseline}$ was defined as the mean $\dot{V}O_2$ measured over the final 90 s of the baseline period. The end-exercise $\dot{V}O_2$ was defined as the mean $\dot{V}O_2$ measured over the final 30 s of exercise.

The mean baseline $\dot{V}CO_2$, $\dot{V}E$, and respiratory exchange ratio (RER) values were calculated over the final 60 s preceding the start of exercise, and the mean end-exercise values were calculated over the final 30 s of exercise.

Muscle Metabolites

Low intensity. To enhance the signal-to-noise ratio of the [PCr], [P_i], [ADP], and [pH] responses, individual subject transitions to low-intensity exercise were time-aligned to the onset of exercise (0 s), averaged, and interpolated generating a single, second-by-second response.

High intensity. To describe the rate of PCr recovery, a time constant was determined by fitting a single-exponential function to the [PCr] measured after the 24-s exercise bout.

Statistical Analyses

Differences in plasma [NO_2^-]; BP; exercise performance; and cardio-respiratory, and muscle metabolic, cognitive function, and brain metabolic responses between the conditions were analyzed with two-tailed, paired-samples *t*-tests, with statistical significance being accepted when $P < 0.05$. Values are expressed as means ± SD.

RESULTS

Twelve participants completed all blood sample, walking exercise, leg extension exercise, and cognitive test sessions. Of the 12 ^{31}P-MRS data sets, 10 were of suitable quality to include in subsequent data analysis. Ten participants completed the ^{1}H-MRS brain scans.

Plasma [NO_2^-] and BP

PL supplementation resulted in no significant change in plasma [NO_2^-] relative to the nonsupplemented control condition. In contrast, BR supplementation elevated plasma [NO_2^-] by 503% relative to control (CON: 206 ± 59 vs. BR: 1,037 ± 627 nM, $P < 0.01$) and by 418% compared with PL (PL: 248 ± 182 nM, $P < 0.01$).

BR supplementation significantly reduced systolic BP relative to control (CON: 125 ± 9 vs. BR: 115 ± 9 mmHg, $P < 0.01$) and compared with PL (120 ± 6 mmHg, $P < 0.05$). Diastolic BP was also significantly reduced with BR ingestion compared with control (CON: 74 ± 7 vs. BR: 70 ± 5 mmHg, $P < 0.01$) and compared with PL (73 ± 5 mmHg, $P < 0.05$). MAP was significantly reduced following BR supplementation relative to both control (CON: 91 ± 7 vs. BR: 85 ± 5 mmHg, $P < 0.01$) and PL (88 ± 4 mmHg, $P < 0.05$).

Moderate-Intensity Walking

The pulmonary $\dot{V}O_2$ responses to a step transition to moderate intensity treadmill exercise in both the PL and BR conditions are presented in Fig. 1, and the parameters derived from the model fit are presented in Table 1. There was no significant difference in $\dot{V}O_2$ between PL and BR during the baseline walking period. The amplitude of the pulmonary $\dot{V}O_2$ response was not different between the two conditions (PL: 477 ± 200 vs. BR: 464 ± 200 ml/min) and the steady-state

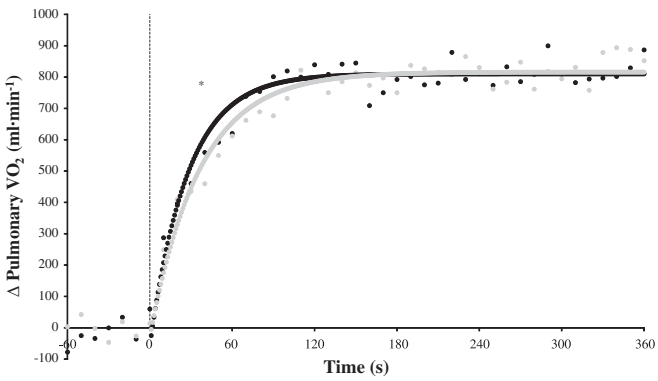

Fig. 1. Pulmonary oxygen uptake ($\dot{V}O_2$) responses during a step increment to a moderate-intensity work rate, following PL and BR supplementation, in a representative subject. Responses following BR are represented by the black line, and responses following PL are represented by the gray line. The dotted vertical line denotes the abrupt "step" transition from baseline to moderate-intensity walking exercise. Data are presented in 10-s intervals.

Table 1. *Pulmonary gas exchange, ventilation, and heart rate during moderate-intensity exercise following placebo and beetroot juice supplementation*

	Placebo	Beetroot
$\dot{V}O_2$		
Baseline, ml/min	518 ± 104	528 ± 87
Primary amplitude, ml/min	477 ± 200	464 ± 200
End exercise, ml/min	979 ± 269	977 ± 250
Mean response time, s	28 ± 7	25 ± 7*
Oxygen deficit, ml	225 ± 132	192 ± 137
$\dot{V}CO_2$		
Baseline, ml/min	452 ± 93	454 ± 73
End exercise, ml/min	847 ± 242	848 ± 200
$\dot{V}E$		
Baseline, l/min	15.2 ± 3.9	15.4 ± 24.9
End exercise, l/min	25.0 ± 7.4	24.9 ± 6.7
Respiratory exchange ratio		
Baseline	0.87 ± 0.06	0.86 ± 0.03
End exercise	0.89 ± 0.05	0.94 ± 0.05
Heart rate		
Baseline, bpm	78 ± 9	77 ± 9
End exercise, bpm	95 ± 12	92 ± 9
Amplitude, bpm	17 ± 12	15 ± 7

Values are expressed as means ± SD. *Significant difference, $P < 0.05$.

$\dot{V}O_2$ measured over the final 30 s of moderate-intensity walking was also unchanged (PL: 979 ± 269 vs. BR: 977 ± 250 ml/min). However, relative to PL, BR supplementation reduced the $\dot{V}O_2$ MRT (PL: 28 ± 7 vs. BR: 25 ± 7 s, $P < 0.05$), and the O_2 deficit (PL: 225 ± 132 vs. BR: 192 ± 137 ml, $P = 0.07$). Baseline and end-exercise $\dot{V}CO_2$, $\dot{V}E$, RER, and HR were not significantly different between conditions (Table 1).

Functional Capacity

Compared to PL, BR did not significantly alter functional capacity as measured by total distance covered in the 6MWT (PL: 667 ± 86 vs. BR: 682 ± 89 m, $P > 0.05$).

Low-Intensity Knee-Extension Exercise

Muscle metabolite concentration changes in response to low-intensity exercise are reported in Table 2 and Fig. 2. There

Table 2. *Muscle metabolic responses during low-intensity exercise following placebo and beetroot juice supplementation*

	Placebo	Beetroot
[PCr]		
Baseline, mM	32.0 ± 5.5	31.9 ± 5.0
240 s, mM	25.8 ± 5.9	26.5 ± 5.8
Amplitude, mM	6.2 ± 2.5	5.3 ± 3.0
[P_i]		
Baseline, mM	3.7 ± 1.0	3.5 ± 0.8
240 s, mM	7.9 ± 1.9	8.3 ± 1.7
Amplitude, mM	4.2 ± 1.1	4.8 ± 1.7
[ADP]		
Baseline, μM	7.4 ± 1.7	7.4 ± 2.1
240 s, μM	22.5 ± 8.4	21.5 ± 8.3
Amplitude, μM	15.1 ± 8.6	14.2 ± 8.5
pH		
Baseline	7.03 ± 0.03	7.02 ± 0.02
240 s	7.07 ± 0.04	7.06 ± 0.02
Δ Baseline – 240 s	0.04 ± 0.02	0.03 ± 0.03

Values are expressed as means ± SD.

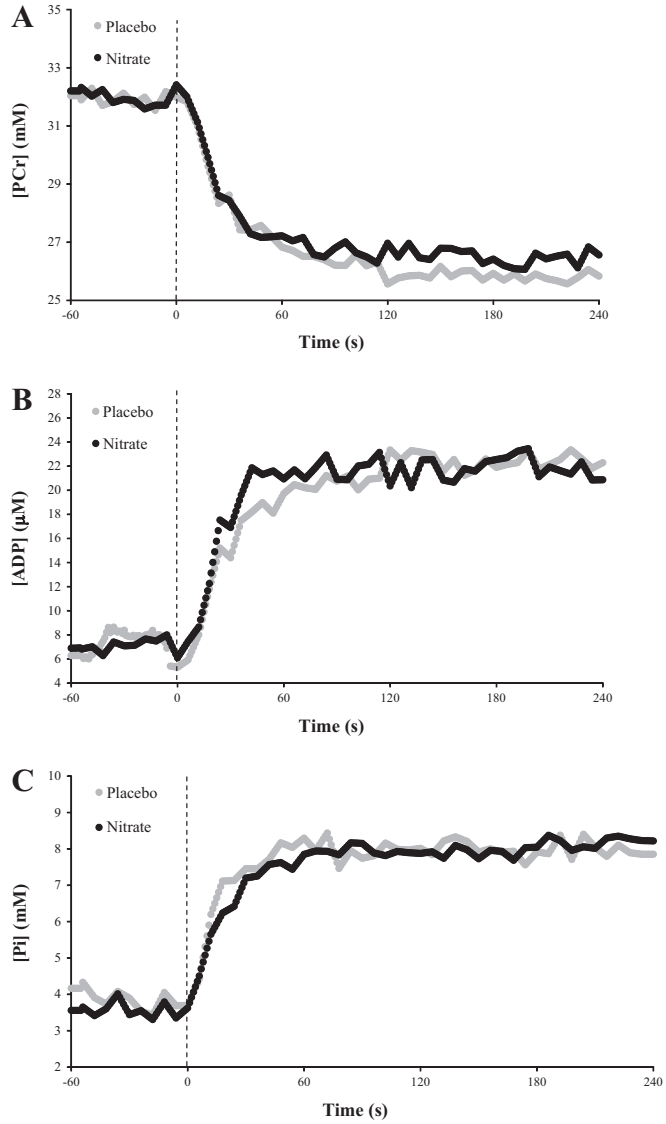

Fig. 2. Group mean muscle metabolic responses to low-intensity, leg-extension exercise following PL and BR supplementation. The change in muscle [PCr] (*A*), [ADP] (*B*), and [P_i] (*C*) from rest to steady state were unaffected by BR supplementation. The dotted vertical line denotes the abrupt "step" transition from rest to low-intensity, leg-extension exercise.

were no significant differences in the baseline or end-exercise [P_i], [ADP], or pH between the two conditions. Although the magnitude of PCr depletion was reduced by ~15% following BR supplementation compared with PL (PL: 6.2 ± 2.5 vs. BR: 5.3 ± 3.0), this difference was not statistically significant.

[PCr] Recovery Kinetics

Muscle metabolite concentration changes in response to the 24-s bout of high-intensity exercise are reported in Table 3, with the PCr depletion and subsequent recovery being illustrated in Fig. 3. Reductions in muscle [PCr], from resting baseline, following high-intensity exercise were not different between the two conditions (PL: 8.1 ± 2.7 vs. BR: 7.3 ± 2.8 mM; $P > 0.05$). The end-exercise pH was also not significantly different from the resting baseline (PL: 7.00 ± 0.03 vs. BR: 7.00 ± 0.03; $P > 0.05$). The [PCr] recovery τ was not different between the two conditions (PL: 35 ± 10 vs. BR: 37 ± 15 s; $P > 0.05$).

Table 3. *Muscle metabolic responses during high-intensity exercise following placebo and beetroot juice supplementation*

	Placebo	Beetroot
[PCr]		
Baseline, mM	30.0 ± 4.2	30.6 ± 5.5
24 s, mM	21.9 ± 4.1	23.2 ± 5.5
Amplitude, mM	8.1 ± 2.7	7.3 ± 2.8
Recovery τ, s	35 ± 10	37 ± 15
[P_i]		
Baseline, mM	2.9 ± 0.9	3.1 ± 1.0
24 s, mM	8.9 ± 2.1	8.9 ± 1.9
Amplitude, mM	6.0 ± 1.7	5.8 ± 1.5
[ADP]		
Baseline, μM	8.0 ± 3.3	8.0 ± 2.2
24 s, μM	37 ± 13.6	34.1 ± 9.4
Amplitude, μM	29.4 ± 12.3	26.1 ± 10.0
pH		
Baseline	6.99 ± 0.03	7.00 ± 0.02
24 s	7.00 ± 0.03	7.00 ± 0.03
Δ Baseline – 240 s	0.01 ± 0.01	0.00 ± 0.04

Values are expressed as means ± SD.

Cognitive Performance

Performance results from the cognitive function tests are presented in Table 4. Cognitive performance on the Serial Subtraction test was not different between PL or BR supplementation for serial 3s (PL: 29 ± 8 vs. BR 26 ± 14, $P > 0.05$) or serial 7s (PL: 16 ± 9 vs. BR: 16 ± 10, $P > 0.05$). Likewise, no significant differences between PL and BR supplementation were found during the Rapid Visual Information Processing test: correct target IDs (PL: 21 ± 4 vs. BR: 23 ± 4, $P > 0.05$), errors (PL: 9 ± 17 vs. BR: 9 ± 16, $P > 0.05$), and average response time (PL: 599 ± 199 vs. BR: 674 ± 194 ms, $P > 0.05$). There were no significant differences in number recall performance data between PL and BR supplementation: forward correct (PL: 29 ± 8 vs. BR: 27 ± 8, $P > 0.05$), backward

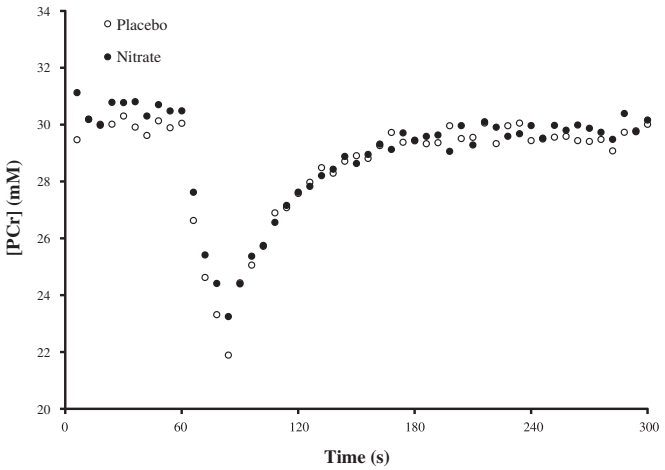

Fig. 3. Group mean intramuscular [PCr] response to 24-s high-intensity, leg-extension exercise and subsequent recovery. [PCr] responses following BR are represented as solid circles, with the PL responses being shown as open circles.

Table 4. *Cognitive performance tests following placebo and beetroot juice supplementation*

	Placebo	Beetroot
Serial subtractions		
3′s, correct responses in 2 min	29 ± 8	26 ± 14
7′s, correct responses in 2 min	16 ± 9	16 ± 10
Rapid visual information processing		
Correct target I.D's	21 ± 4	23 ± 4
Errors	9 ± 17	9 ± 16
Average response time, ms	599 ± 199	674 ± 194
Number recall		
Forwards correct	29 ± 8	27 ± 8
Backwards correct	22 ± 7	21 ± 7
Total correct	51 ± 14	48 ± 14

Values are expressed as means ± SD.

correct (PL: 22 ± 7 vs. BR: 21 ± 7, $P > 0.05$) and total correct (PL: 51 ± 14 vs. BR: 48 ± 14, $P > 0.05$).

Brain Metabolic Concentrations

A summary of the effects of BR supplementation upon resting brain metabolite concentrations and apparent diffusion coefficients is presented in Table 5. Resting concentration ratios of NAA:water, Cr:water, Ch:water, mL:water, NAA:Cr, NAA:Ch, and NAA:Cr+Ch in both left frontal white matter and occipito-parietal gray matter were not significantly different between the two conditions. Likewise, there were no differences between PL and BR in apparent diffusion coefficients from the anterior cingulate gyrus, the dorsolateral prefrontal cortex, and the subcortical white matter of the frontal lobe, suggesting BR did not modulate diffusive characteristics in the brain.

DISCUSSION

The principal original findings of this investigation were that, consistent with our hypotheses, short-term (2.5 days) dietary NO_3^- supplementation in the form of concentrated beetroot juice (which elevated plasma [nitrite] four-fold) significantly reduced resting blood pressure and the $\dot{V}O_2$ mean response time during walking exercise in a healthy senescent population. These findings are important, as they provide evidence that dietary supplementation with a natural food product may act as a valuable intervention in preventing hypertension and speeding $\dot{V}O_2$ kinetics in older adults. However, in contrast to our hypotheses, NO_3^- supplementation did not significantly alter the steady-state O_2 cost of walking, functional walking performance, the muscle metabolic response to low-intensity exercise, brain metabolite concentrations, or cognitive function.

Effects of Nitrate Supplementation on Plasma [NO_2^-] and BP

Following supplementation with NO_3^--rich BR, plasma [NO_2^-] was increased to 418% of the PL value. These findings are consistent with previous studies that reported significant elevations in plasma [NO_2^-] following dietary NO_3^- supplementation (29, 50, 76). CON plasma [nitrite] values in the present study population were similar to those found in young adults. This was surprising because lower [nitrite] values may be expected in an older population (68). Moreover, it might be expected that the effect of NO_3^- supplementation on plasma [NO_2^-] might be smaller in older compared with younger adults due to age-related changes in oral bacterial colonization (63). However, the elevation of plasma [NO_2^-] in the current study was somewhat greater than that found in previous research with younger adults (4, 29, 50, 76, 81) but similar to that reported previously in older healthy subjects (57) and peripheral arterial disease patients (41).

It is possible that increased plasma [NO_2^-] might augment NO bioavailability, thereby compensating for the expected age-dependent reduction in endothelial NOS activity (68). Increased extracellular NO promotes smooth muscle relaxation via the synthesis of cyclic guanosine monophosphate from guanosine triphosphate. Previous studies have revealed significant reductions in systolic and diastolic BP as a result of this NO-related smooth muscle relaxation (49, 81). Likewise, in the present study, we found significant reductions in systolic blood pressure (−5 mmHg), diastolic blood pressure (−3 mmHg), and mean arterial pressure (−3 mmHg) following ingestion of the NO_3^--rich BR, relative to the NO_3^--depleted PL. Supplementation with the NO_3^--depleted PL did not significantly reduce diastolic BP or mean arterial pressure relative to the control condition, which may suggest that the NO_3^- in BR, rather than other compounds found in BR, including antioxidants (83), were principally responsible for the lowering of resting BP. On the other hand, PL did have a small but significant effect on systolic BP relative to control, which may indicate that NO_3^- is not the only bioactive compound in BR, which contributes to the lowering of BP. The present study indicates that BP can be reduced via the systemic reduction of NO_3^--derived NO_2^- in healthy older adults in a similar fashion to that reported previously in young adults (81). This finding is in contrast to a recent study in which dietary nitrate supplementation increased plasma nitrate and nitrite values but did not alter BP in older adults (57). The reason for this difference

Table 5. *^{1}H-MRS and ADC brain scan data following placebo and beetroot juice supplementation*

^{1}H-MRS Brain Scans	Placebo	Beetroot
Gray matter		
NAA:Water	2.065 ± 0.273	2.141 ± 0.213
Cr:Water	1.024 ± 0.111	1.050 ± 0.090
Ch:Water	0.568 ± 0.117	0.559 ± 0.085
mI:Water	0.757 ± 0.193	0.803 ± 0.102
NAA:Cr	2.031 ± 0.294	2.048 ± 0.245
NAA:Ch	3.805 ± 1.054	3.908 ± 0.696
NAA:Cr+Ch	1.315 ± 0.235	1.337 ± 0.162
White matter		
NAA:Water	1.575 ± 0.263	1.637 ± 0.180
Cr:Water	0.895 ± 0.140	0.940 ± 0.078
Ch:Water	1.016 ± 0.108	0.992 ± 0.149
mI:Water	0.950 ± 0.226	1.065 ± 0.337
NAA:Cr	1.761 ± 0.118	1.742 ± 0.143
NAA:Ch	1.546 ± 0.177	1.692 ± 0.401
NAA:Cr+Ch	0.821 ± 0.069	0.850 ± 0.109
ADC, 10^{-3}		
Dorsolateral, prefrontal cortex	0.782 ± 0.033	0.783 ± 0.050
Anterior cingulated gyrus	0.753 ± 0.137	0.790 ± 0.101
Frontal lobe (deep white matter)	0.817 ± 0.052	0.841 ± 0.073

Values are expressed as means ± SD. MRS, magnetic resonance spectroscopy; NAA, *N*-acetyl aspartate; Cr, creatine; Ch, choline; mI, myo-inositol; ADC, apparent diffusion coefficient.

is unclear. Our results suggest that a high NO_3^- diet may benefit cardiovascular health in older adults.

Effects of Nitrate Supplementation on the Physiological Responses to Walking

A novel finding of the present study was the small but significant speeding of $\dot{V}O_2$ kinetics following the onset of exercise subsequent to dietary NO_3^- supplementation. Faster $\dot{V}O_2$ kinetics would be expected to reduce the reliance on nonoxidative metabolic processes across the transition from a lower to a higher metabolic rate and, therefore, to reduce muscle metabolic perturbation (i.e., changes in substrates or metabolites that have been associated with fatigue development; Ref. 36, 82). In the present study, the O_2 deficit was reduced by 15% following NO_3^- supplementation, as a function of the faster $\dot{V}O_2$ kinetics. Whether the small speeding of $\dot{V}O_2$ kinetics that we observed is of functional relevance remains unclear, however, given that we did not find differences in 6MWT performance. Previous studies with young adults have not found faster $\dot{V}O_2$ kinetics following NO_3^- supplementation (5, 47, 76). Older adults typically have slower $\dot{V}O_2$ kinetics (3, 14, 22) and are more likely to evince a speeding of $\dot{V}O_2$ kinetics following interventions designed to enhance muscle O_2 delivery (66) than their younger counterparts. The MRT for $\dot{V}O_2$ kinetics for the older subjects tested in the present study was surprisingly fast (i.e., ~28 s). This may be due to both the exercise modality that we employed (i.e., walking) and the fact that our subjects were physically active. Given that NO_3^- supplementation did not significantly alter the maximal rate of oxidative ATP resynthesis (see *Effects of Nitrate Supplementation on Muscle [PCr] Recovery*), it is possible that the faster $\dot{V}O_2$ kinetics that we observed was linked to enhanced muscle vasodilatation and blood flow, which offset an O_2 delivery limitation to $\dot{V}O_2$ kinetics in our older subjects.

No effects on the O_2 cost of walking were evident in the present study, which is in contrast to results reported recently in younger adults (47) and to the body of literature, which indicates that NO_3^- supplementation improves exercise efficiency (4, 51, 76). It is unclear why older adults may respond differently to younger adults with respect to the influence of NO_3^- supplementation on the O_2 cost of exercise. However, the lack of significant change in walking economy is consistent with the lack of change in muscle metabolic responses that we observed (see *Effects of Nitrate Supplementation on Muscle Metabolism During Low-Intensity Exercise*).

Effects of Nitrate Supplementation on Functional Capacity

Dietary NO_3^- supplementation has been reported to improve high-intensity exercise tolerance (4, 5, 47), and time-trial performance (12, 48) in athletic young adults. In the present study, we assessed the functional capacity of our older subjects using the 6MWT. There was no significant difference in 6MWT performance between PL and BR. However, there was a 2.2% mean increase in total distance covered in the BR condition, which is similar to the improvements in performance reported for 4 km and 16.1 km (~2.7%; Ref. 48) and 10 km (~1.0%; Ref. 12) cycling time-trials. A speeding of the $\dot{V}O_2$ kinetics, as was observed in the present study following NO_3^- supplementation, would be expected to improve performance in certain physical tasks. It is unclear why NO_3^- supplementation did not result in a significant improvement in 6MWT performance in the present study. It is possible that the 6MWT lacks the sensitivity to detect small improvements in functional capacity consequent to an acute intervention (24), especially in the physically active subjects in the present study. Future investigations into the influence of NO_3^- supplementation on functional capacity in older adults might usefully employ a more comprehensive battery of physical performance tests.

Effects of Nitrate Supplementation on Muscle Metabolism During Low-Intensity Exercise

In the present study, the fall in muscle [PCr] during low-intensity knee-extensor exercise was not significantly attenuated following NO_3^- supplementation. However, the magnitude of [PCr] depletion was reduced by 15%, on average. In an earlier study in young adults we reported that NO_3^- supplementation significantly reduced the amplitude of [PCr] depletion during low-intensity exercise (5). The linear relationship observed between $\dot{V}O_2$ and intramuscular [PCr], both before and after NO_3^- supplementation, suggested that the reduction in the O_2 cost of exercise was subsequent to enhanced efficiency within the muscle contractile apparatus. It is unclear why the fall in muscle [PCr] was significantly spared in younger adults (5) but not older adults (present study). Interindividual variability may have precluded the attainment of statistical significance in the present study. Alternatively, the lower ATP cost of muscle contraction in older adults (74) may have served to reduce the impact of NO_3^- supplementation on muscle contractile efficiency.

Effects of Nitrate Supplementation on Muscle [PCr] Recovery

The rate at which intramuscular [PCr] recovers immediately following exercise is thought to reflect the maximal rate of oxidative synthesis of ATP, with limited contribution from glycolysis (38). An increased rate of [PCr] recovery would suggest improvements in maximal oxidative rate as a function of increased mitochondrial volume and/or oxidative enzyme activity or, in the event of tissue hypoxia, O_2 supply (1). In the present study, NO_3^- supplementation did not significantly alter muscle [PCr] recovery kinetics, consistent with our previous findings in young adults (47).

Effects of Nitrate Supplementation on Brain Metabolite Concentrations and Cognitive Performance

The amino acid *N*-acetylaspartate (NAA) found in neurons in the adult central nervous system (58) has been suggested to be a marker of neuronal viability (54). NAA has been shown to be closely related to mitochondrial activity in ATP production and O_2 consumption (6), which suggests an association between [NAA] and metabolic efficiency in the brain (73). Previous studies have shown that [NAA] is associated with both intellectual and neuropsychological (84) measures of cognition in young adults. In the present study, we considered whether NO_3^- supplementation may provide beneficial effects upon metabolic efficiency and blood flow within the brain, in a similar fashion to what has been reported within skeletal muscle (50, 77). However, there were no significant differences in [NAA] following NO_3^- supplementation. Likewise,

mI, a carbohydrate found in the brain that is elevated in patients with Alzheimer's disease and mild cognitive impairment (33, 43), was not affected by the NO_3^- supplementation. Moreover, NO_3^- supplementation did not alter the concentrations of Cr or Ch in the brain, both of which are considered important in neurological health, energy metabolism, and cognitive ability (56, 78). It is well documented that chronic ischemia and poor cerebral perfusion, specifically to the white matter, is associated with cognitive decline and dementia (69). It was recently shown that an elevated dietary NO_3^- intake increased cerebral blood flow to the anterior cingulated gyrus, the dorsolateral prefrontal cortex and subcortical and deep white matter of the frontal lobes in a population of older adults (63). We were unable to identify changes to apparent diffusion coefficients in the aforementioned regions despite providing a larger NO_3^- dose to our subjects (24.6 mmol over 2.5 days) compared with Presley et al. (63) (12.4 mmol over 2 days). A possible explanation for this discrepancy is that the subjects in the Presley et al. study (63) were, on average, 10 yr older than the subjects we studied, increasing the likelihood that blood flow to these specific brain areas was diminished.

Given the previous report that increased dietary NO_3^- intake increased brain blood flow in older adults (63), we assessed the influence of NO_3^- supplementation on cognitive function. Specifically, measures of attention, concentration, information processing, and working memory were completed using validated cognitive function tests. However, we could not discern significant effects of NO_3^- supplementation on cognitive function. A lack of effect of NO_3^- supplementation on cognitive function might not be considered surprising given that there were no significant changes in NMR parameters of cerebral functionality or metabolism.

Experimental Considerations

Although we have attributed the reductions in resting BP and $\dot{V}O_2$ MRT during the transition to walking exercise to an increased NO_3^- intake, we appreciate that BR contains a number of other compounds that may influence physiological function in humans at rest and during exercise. Specifically, betaine has been linked to improving muscular endurance, strength, and power (31, 55) and can be found in beetroot. Likewise, the polyphenols, quercetin and resveratrol, which are found in beetroot have, in some instances, been reported to increase aerobic capacity and stimulate mitochondrial biogenesis (20, 44). Although we do not rule out the potential for NO_3^- to operate synergistically with these compounds, the unchanged plasma [NO_2^-], diastolic BP, MAP, and $\dot{V}O_2$ response following PL supplementation suggests that NO_3^- is the key "bioactive" compound in BR. Nevertheless, the reduced systolic BP following PL supplementation compared with the control condition may suggest that other components of beetroot juice, such as antioxidants (83), might also contribute to the BP-lowering effect of BR in older adults.

While we were successful in recruiting a cohort of older adults to this study (mean age of 64 yr), the subjects tended to be physically active and were interested in the health benefits of diet and exercise. In this regard, they may have been unrepresentative of their age group (for example, they had a very fast $\dot{V}O_2$ MRT), and this may have reduced the likely impact of NO_3^- supplementation on functional capacity assessed with the 6MWT, regional brain blood flow, and cognitive function. That is, there may have been limited opportunity for NO_3^- supplementation to positively influence physical or cognitive function because our subjects were not yet sufficiently impaired. Moreover, the 6MWT might not have been the most sensitive or appropriate test for these physically fit older adults. Physical and cognitive decline is likely accelerated beyond ~70 yr of age (46, 69), and our results do not discount the possibility that NO_3^- supplementation may be beneficial in older, more impaired individuals (e.g., Ref. 63). It is also pertinent to note that the dietary intervention in the present study was short-term. Longer-term NO_3^- supplementation may be required to enhance vascular structure and function (68), which may, in turn, improve the matching of O_2 delivery to metabolic rate (7, 18) and enhance metabolic control. Future studies should consider the possible benefits of longer-term NO_3^- supplementation in senescent subjects with greater physical and cognitive impairment.

Perspectives and Significance

Short-term (2.5 days) dietary NO_3^- supplementation resulted in a four-fold increase in plasma [nitrite] and significant reductions in resting blood pressure in normotensive older adults. These results suggest that NO_3^- supplementation may have potential in reducing the risk of hypertension and cardiovascular disease in older adults. The $\dot{V}O_2$ kinetics was accelerated during treadmill walking, although this did not translate into enhanced performance during a 6MWT. Indices of brain metabolism and cognitive performance were not significantly altered. The results suggest that increased dietary NO_3^- intake may provide a practical therapeutic and/or prophylactic intervention for reducing the risk of hypertension and improving $\dot{V}O_2$ kinetics in older adults. Whether this may translate into improved functional capacity in functionally impaired older adults should be considered in subsequent research.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: J.K., J.F., A.V., S.J.B., M.G., P.G.W., and A.M.J. conception and design of research; J.K., J.F., and O.F. performed experiments; J.K., J.F., J.R.B., O.F., and P.G.W. analyzed data; J.K., J.F., A.V., J.R.B., S.J.B., P.G.W., and A.M.J. interpreted results of experiments; J.K. prepared figures; J.K., O.F., and S.J.B. drafted manuscript; J.K., J.F., A.V., S.J.B., M.G., P.G.W., and A.M.J. edited and revised manuscript; J.K., J.F., A.V., J.R.B., O.F., S.J.B., M.G., P.G.W., and A.M.J. approved final version of manuscript.

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