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Dietary nitrate supplementation reduces the O_2 cost of low-intensity exercise and enhances tolerance to high-intensity exercise in humans

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Bailey SJ, Winyard P, Vanhatalo A, Blackwell JR, DiMenna FJ, Wilkerson DP, Tarr J, Benjamin N, Jones AM. Dietary nitrate supplementation reduces the O_2 cost of low-intensity exercise and enhances tolerance to high-intensity exercise in humans. *J Appl Physiol* 107: 1144–1155, 2009. First published August 6, 2009; doi:10.1152/japplphysiol.00722.2009.—Pharmacological sodium nitrate supplementation has been reported to reduce the O_2 cost of submaximal exercise in humans. In this study, we hypothesized that dietary supplementation with inorganic nitrate in the form of beetroot juice (BR) would reduce the O_2 cost of submaximal exercise and enhance the tolerance to high-intensity exercise. In a double-blind, placebo (PL)-controlled, crossover study, eight men (aged 19–38 yr) consumed 500 ml/day of either BR (containing 11.2 ± 0.6 mM of nitrate) or blackcurrant cordial (as a PL, with negligible nitrate content) for 6 consecutive days and completed a series of "step" moderate-intensity and severe-intensity exercise tests on the last 3 days. On *days 4–6*, plasma nitrite concentration was significantly greater following dietary nitrate supplementation compared with PL (BR: 273 ± 44 vs. PL: 140 ± 50 nM; $P < 0.05$), and systolic blood pressure was significantly reduced (BR: 124 ± 2 vs. PL: 132 ± 5 mmHg; $P < 0.01$). During moderate exercise, nitrate supplementation reduced muscle fractional O_2 extraction (as estimated using near-infrared spectroscopy). The gain of the increase in pulmonary O_2 uptake following the onset of moderate exercise was reduced by 19% in the BR condition (BR: 8.6 ± 0.7 vs. PL: 10.8 ± 1.6 $ml \cdot min^{-1} \cdot W^{-1}$; $P < 0.05$). During severe exercise, the O_2 uptake slow component was reduced (BR: 0.57 ± 0.20 vs. PL: 0.74 ± 0.24 l/min; $P < 0.05$), and the time-to-exhaustion was extended (BR: 675 ± 203 vs. PL: 583 ± 145 s; $P < 0.05$). The reduced O_2 cost of exercise following increased dietary nitrate intake has important implications for our understanding of the factors that regulate mitochondrial respiration and muscle contractile energetics in humans.

exercise economy; muscle efficiency; O_2 uptake; exercise performance; fatigue

A FUNDAMENTAL TENET OF HUMAN exercise physiology is a predictable oxygen (O_2) cost for a given submaximal work rate. Upon the initiation of moderate-intensity exercise [i.e., exercise performed at work rates below the gas exchange threshold (GET)], pulmonary O_2 uptake ($\dot{V}O_2$), which closely reflects O_2 consumption in the skeletal muscles (2, 29, 38), rises in an exponential fashion to attain a "steady state" within ~2–3 min in healthy humans (64). The steady-state increase in $\dot{V}O_2$ is linearly related to the increase in external work rate; is essentially independent of factors such as age, health status or aerobic fitness; and approximates 10 ml $O_2 \cdot min^{-1} \cdot W^{-1}$ of external power output during cycle ergometry (i.e., 10 $ml \cdot min^{-1} \cdot W^{-1}$; Ref. 36). During supra-GET exercise, $\dot{V}O_2$ dynamics become more complex, owing, in part, to the development of a delayed-onset $\dot{V}O_2$ "slow component", which elevates the O_2 cost of exercise above 10 $ml \cdot min^{-1} \cdot W^{-1}$ (36, 64).

Whereas it is known that interventions such as training and the inspiration of hyperoxic gas can reduce the O_2 cost of heavy (above the GET but below critical power; Ref. 52) and severe (above critical power) exercise by reducing the amplitude of the $\dot{V}O_2$ slow component, the steady-state $\dot{V}O_2$ during moderate exercise is unaffected by these and other interventions in healthy humans (1, 15, 36, 51, 65). Surprisingly, however, it was recently reported that 6 days of dietary supplementation with pharmacological sodium nitrate reduced the O_2 cost of submaximal cycling at work rates expected to require 45–80% maximum $\dot{V}O_2$ ($\dot{V}O_{2max}$) (45). That this effect occurred without any increase in estimated nonoxidative energy production (as reflected by an unchanged blood [lactate]) (where brackets denote concentration) suggested that sodium nitrate ingestion improved the efficiency of muscle oxidative metabolism. It is known that tolerance to high-intensity exercise is, in certain respects, a function of $\dot{V}O_{2max}$ and submaximal exercise economy (20). Therefore, assuming that $\dot{V}O_{2max}$ is not altered, it is feasible that dietary nitrate supplementation might enhance exercise tolerance. However, this possibility has not been investigated.

The nitrate anion (NO_3^-) is relatively inert, and thus any biological effects are likely conferred via its conversion to the bioactive nitrite anion (NO_2^-). Inorganic nitrate is rapidly absorbed from the gut and is concentrated in saliva at least 10-fold. In the mouth, facultative anaerobic bacteria on the surface of the tongue reduce NO_3^- to NO_2^- (23). Nitrite can be converted to nitric oxide (NO) in the stomach (6, 47), but it is also clear that some is absorbed to increase circulating plasma [nitrite] (21, 46). We and several other groups have shown that NO_2^- can be converted to NO under appropriate physiological conditions (6). The requisite one-electron reduction has variously been reported to be catalyzed via xanthine oxidoreductase, hemoglobin, myoglobin, endothelial NO synthase, and the mitochondrial electron transfer complexes (61).

There are at least two mechanisms by which NO derived from NO_2^- (rather than from the much better known synthesis of NO from L-arginine by the NO synthase family of enzymes) might influence O_2 utilization by contracting skeletal muscle. First, as all of the known mechanisms for NO_2^- reduction are facilitated by hypoxia, it may be that more NO (which is a potent vasodilator) is generated in parts of muscle that are receiving less or using more O_2, and, therefore, this mechanism would help to match local blood flow to O_2 requirement, providing a more homogenous distribution of O_2 within skel-

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etal muscle. However, while this might be beneficial in terms of muscle function, it would not explain a reduced O_2 cost during exercise. A second possible mechanism involves the roles of NO_2^- and NO as regulators of cellular O_2 utilization. For example, NO is known to be an important inhibitor of cytochrome oxidase activity (10). More recently, it has been suggested that NO might enhance the efficiency of oxidative phosphorylation by reducing "slippage" of the mitochondrial proton pumps (17). There is also evidence that NO_2^- can serve as an alternative electron acceptor, theoretically replacing the role of O_2 in respiration (3).

The diet constitutes the main source of NO_3^- in humans, with vegetables accounting for 60–80% of daily NO_3^- intake in a Western diet (67). Given the reported ability of pharmacological sodium nitrate to reduce the O_2 cost of exercise (45), we sought to determine whether similar effects are observed when the NO_3^- dose is administered in the form of nitrate-rich beetroot juice (BR). This is important because sodium nitrate is a pharmaceutical product, whereas BR is a natural food product that can be readily ingested as part of the normal diet. We, therefore, investigated the influence of BR ingestion on plasma [nitrite], blood pressure (BP), muscle oxygenation [assessed with near-infrared spectroscopy (NIRS)] and the $\dot{V}O_2$ response to step transitions to moderate- and severe-intensity exercise. We hypothesized that dietary BR supplementation would reduce the O_2 cost of moderate-intensity exercise and increase exercise tolerance (assessed as the time-to-task failure) during severe-intensity exercise.

METHODS

Subjects

Eight healthy men (mean ± SD, age 26 ± 7 yr, height 180 ± 3 cm, body mass 82 ± 6 kg; $\dot{V}O_{2max}$ 49 ± 5 ml·kg^{-1}·min^{-1}) who were recreationally active in sporting activities volunteered to participate in this study. None of the subjects was a tobacco smoker or user of dietary supplements. All subjects were fully familiar with laboratory exercise testing procedures, having previously participated in studies employing cycle ergometry in our laboratory. The procedures employed in this study were approved by the Institutional Research Ethics Committee. All subjects gave their written, informed consent before the commencement of the study, after the experimental procedures, associated risks, and potential benefits of participation had been explained. 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. Each subject was also asked to refrain from caffeine and alcohol intake 6 and 24 h before each test, respectively. All tests were performed at the same time of day (±2 h).

Procedures

The subjects were required to report to the laboratory on seven occasions, over a 4- to 5-wk period. During the first visit to the laboratory, subjects performed a ramp incremental exercise test for determination of the peak $\dot{V}O_2$ ($\dot{V}O_{2\,peak}$) and GET. All cycle tests were performed on an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, the Netherlands). Initially, subjects completed 3 min of "unloaded" baseline cycling, after which the work rate was increased at a rate of 30 W/min until the subject was unable to continue. The participants cycled at a self-selected pedal rate (70–90 rpm), and this pedal rate, along with saddle and handle bar height and configuration, was recorded and reproduced in subsequent tests. The breath-by-breath pulmonary gas-exchange data were collected continuously during the incremental tests and averaged over consecutive 10-s periods. The $\dot{V}O_{2\,peak}$ was taken as the highest 30-s average value attained before the subject's volitional exhaustion. The GET was determined as described previously (1, 5). The work rates that would require 80% of the GET (moderate exercise) and 70% Δ (70% of the difference between the power output at the GET and $\dot{V}O_{2\,peak}$, severe exercise) were subsequently 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 power output at GET and peak; Ref. 63).

Following completion of the ramp test, subjects were randomly assigned in a crossover design, to receive 6 days of dietary supplementation with either nitrate (NO_3^-; 5.5 mmol/day; administered as 0.5 liter organic BR/day; Beet It, James White Drinks, Ipswich, UK) or "placebo" (PL; low-calorie black currant juice cordial with negligible nitrate content). The subjects were instructed to sip the beverages at regular intervals throughout the day. A 10-day washout separated the supplementation periods. The order between the nitrate and PL supplementation periods was balanced. The subjects were provided with a list of foods rich in nitrates and asked to abstain from consuming these foods for the duration of the study. The subjects were not aware of the experimental hypotheses to be tested but were informed that the purpose of the study was to compare the physiological responses to exercise following the consumption of two commercially available beverages. The personnel administering the exercise tests were not aware of the type of beverage being consumed by the subjects.

On *days 4*, *5*, and *6* of the supplementation periods, the subjects completed "step" exercise tests from a 20-W baseline to moderate- and severe-intensity work rates for the determination of pulmonary $\dot{V}O_2$ dynamics. On the 4th day of supplementation, subjects completed two bouts of moderate cycling, while on *days 5* and *6* the subjects completed one bout of moderate cycling and one bout of severe cycling. The two bouts of exercise on each day were separated by 25 min of passive recovery. All exercise bouts were of 6-min duration, with the exception of the severe exercise bout on the final day, which was continued until task failure as a measure of exercise tolerance. The time to task failure was recorded when the pedal rate fell by >10 rpm below the self-selected pedal rate. In these bouts, the subjects were verbally encouraged to continue for as long as possible. The $\dot{V}O_2$ responses to the four moderate and two severe exercise bouts were averaged before analysis to reduce breath-to-breath noise and enhance confidence in the parameters derived from the modeling process (44). Before each exercise bout, BP was measured, and venous blood samples were collected for subsequent determination of plasma [nitrite] (see Measurements below).

Measurements

During all tests, pulmonary gas exchange and ventilation were measured continuously using a portable metabolic cart (MetaMax 3B, Cortex Biophysik, Leipzig, Germany), as described previously (1). A turbine digital transducer measured inspired and expired airflow, while an electrochemical cell O_2 analyzer and an infrared CO_2 analyzer simultaneously measured expired gases. Subjects wore a nose clip and breathed through a low-dead-space, low-resistance mouthpiece that was securely attached to the volume transducer. The inspired and expired gas volume and gas concentration signals were continuously sampled via a capillary line connected to the mouthpiece and displayed breath by breath. Heart rate (HR) was measured during all tests using short-range radiotelemetry (Polar S610, Polar Electro Oy, Kempele, Finland). During one of the transitions to moderate and severe exercise, for both supplementation periods, a blood sample was collected from a fingertip into a capillary tube over the 20 s preceding the step transition in work rate and within the last 20 s of exercise. A capillary blood sample was also collected at the limit of tolerance for the severe bout performed on *day 6* of each supplementation period.

These whole blood samples were subsequently analyzed to determine blood [lactate] (YSI 1500, Yellow Springs Instruments, Yellow Springs, OH) within 30 s of collection. Blood lactate accumulation (Δblood [lactate]) was calculated as the difference between blood [lactate] at end-exercise and blood [lactate] at baseline.

The oxygenation status of the *m. vastus lateralis* of the right leg was monitored using a commercially available NIRS system (model NIRO 300, Hamamatsu Photonics KK, Hiugashi-ku, Japan). The system consisted of an emission probe that irradiates laser beams and a detection probe. Four different wavelength laser diodes provided the light source (776, 826, 845, and 905 nm), and the light returning from the tissue was detected by a photomultiplier tube in the spectrometer. The intensity of incident and transmitted light was recorded continuously at 2 Hz and used to estimate concentration changes from the resting baseline for oxygenated, deoxygenated, and total tissue hemoglobin/myoglobin. Therefore, the NIRS data represent a relative change based on the optical density measured in the first datum collected. The deoxyhemoglobin concentration ([HHb]) signal can be regarded as being essentially blood-volume insensitive during exercise and so was assumed to reflect the balance between local O_2 supply and utilization and to provide an estimate of changes in O_2 extraction in the field of interrogation (1, 22, 24, 30). The leg was initially cleaned and shaved around the belly of the muscle, and the probes were placed in the holder, which was secured to the skin with adhesive at 20 cm above the fibular head. To secure the holder and wires in place, an elastic bandage was wrapped around the subject's leg. The wrap helped to minimize the possibility that extraneous light could influence the signal and also ensured that the optodes did not move during exercise. Pen marks were made around the probes to enable precise reproduction of the placement in subsequent tests. The probe gain was set with the subject at rest in a seated position with the leg extended at down stroke on the cycle ergometer before the first exercise bout, and NIRS data were collected continuously throughout the exercise protocols. The data were subsequently downloaded onto a personal computer, and the resulting text files were stored on disk for later analysis.

BP of the brachial artery was measured with subjects in a rested, seated position before each exercise bout using an automated sphygmomanometer. Three measurements were taken at each sample point with the mean of the second and third BP measurements being recorded. Venous blood samples were also drawn into lithium-heparin tubes before each exercise bout and centrifuged at 4,000 rpm and 4°C for 10 min, within 3 min of collection. Plasma was subsequently extracted and immediately frozen at −80°C for later analysis of NO_2^- via chemiluminescence (4).

All glass wear, utensils, and surfaces were rinsed with deionized water to remove residual NO_2^- before analysis. After thawing at room temperature, plasma samples were initially deproteinized before analysis using the procedures of Higuchi and Motomizu (33). Initially, 100 μl of sample were placed in a microcentrifuge tube, along with 200 μl of deionized H_2O and 300 μl of 0.3 N NaOH, and left to stand at room temperature for 5 min. Then 300 μl of 5% by weight aqueous $ZnSO_4$ was added to the mixture, after which the sample was vortexed and left to stand at room temperature for a further 10 min. Thereafter, samples were centrifuged at 4,000 rpm for 15 min, and the supernatant was removed for subsequent analysis. The $[NO_2^-]$ of the deproteinized plasma samples was determined by its reduction to NO in the presence of 5-ml glacial acetic acid and 1% NaI under nitrogen at room temperature in a gas-sealed purging vessel. Samples were introduced to the vessel via injection into the septum at the top of the vessel. The NO content was quantified by a chemiluminescence NO analyzer (Sievers, 280i NO analyzer). The reaction of NO with ozone in the chemiluminescent reaction chamber yielded electronically excited NO_2 (nitrogen dioxide), which emits light at the infrared region of the electromagnetic spectrum. Ozone was generated from an O_2 supply via an electrostatic ozone generator and high-voltage transformer. To minimize the interference of the chemiluminescent reactions of sulfur-containing compounds, an optical filter transmitted only red wavelengths (>600 nm), since the light emitted by sulfur-containing compounds is of shorter wavelengths. The intensity of the filtered infrared light was quantified by a red-sensitive photomultiplier tube and amplified producing an analog millivolt output signal. The $[NO_2^-]$ was derived from the integral of the NO-generated millivolt signal over time compared with those obtained for $NaNO_2^-$ standards.

Data Analysis Procedures

The breath-by-breath $\dot{V}O_2$ data from each test were initially examined to exclude errant breaths caused by coughing, swallowing, sighing, etc., and those values lying more than 4 SDs from the local mean were 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. The two severe exercise bouts were of different duration (6 min for the first bout and >6 min in the second bout, which was performed to task failure), and so, at this intensity, only the first 6 min of data were averaged together and modeled. The first 20 s of data after the onset of exercise (i.e., the phase I response) were deleted, and a nonlinear least squares algorithm was used to fit the data thereafter. A single-exponential model was used to characterize the $\dot{V}O_2$ responses to moderate exercise, and a biexponential model was used for severe exercise, as described in the following equations:

$$\dot{V}O_2(t) = \dot{V}O_{2\,\text{baseline}} + A_p\left[1 - e^{-(t-TD_p/\tau_p)}\right] \quad \text{(moderate)} \qquad (1)$$

$$\dot{V}O_2(t) = \dot{V}O_{2\,\text{baseline}} + A_p\left[1 - e^{-(t-TD_p/\tau_p)}\right] + A_s\left[1 - e^{-(t-TD_s/\tau_s)}\right] \quad \text{(severe)} \qquad (2)$$

where $\dot{V}O_2$ (t) represents the absolute $\dot{V}O_2$ at a given time t; $\dot{V}O_{2\text{baseline}}$ represents the mean $\dot{V}O_2$ in the baseline period; A_p, TD_p, and τ_p represent the amplitude, time delay, and time constant, respectively, describing the phase II increase in $\dot{V}O_2$ above baseline; and A_s, TD_s, and τ_s represent the amplitude of, time delay before the onset of, and time constant describing the development of the $\dot{V}O_2$ slow component, respectively.

An iterative process was used to minimize the sum of the squared errors between the fitted function and the observed values. $\dot{V}O_{2\text{baseline}}$ was defined as the mean $\dot{V}O_2$ measured over the final 90 s of baseline pedaling. The end-exercise $\dot{V}O_2$ was defined as the mean $\dot{V}O_2$ measured over the final 30 s of exercise. Because the asymptotic value (A_s) of the exponential term describing the $\dot{V}O_2$ slow component may represent a higher value than is reached at the end of the exercise, the actual amplitude of the $\dot{V}O_2$ slow component at the end of exercise was defined as A_s'. The A_s' parameter was compared at the same iso-time (360 s) under both supplementation periods. The amplitude of the slow component was also described relative to the entire $\dot{V}O_2$ response. In addition, the functional "gain" of the fundamental $\dot{V}O_2$ response was computed by dividing A_p by the Δwork rate. The functional gain of the entire response (i.e., end-exercise gain) was calculated in a similar manner. To determine the overall kinetics of the $\dot{V}O_2$ response to both moderate- and severe-intensity exercise, data were fit with a monoexponential model from 0 s to end exercise, without TD.

To provide information on muscle oxygenation, we also modeled the [HHb] response to exercise. Mono- and biexponential models, similar to those described above, were applied to the ensemble-averaged data, with the exception that the fitting window commenced at the time at which the [HHb] signal increased 1 SD above the baseline mean (22). The [HHb] kinetics for moderate exercise were determined by constraining the fitting window to the point at which monoexponentiality became distorted, consequent to a gradual fall in [HHb] (1), as determined by visual inspection of the residual plots. The [HHb] kinetics for severe exercise were determined by fitting a biexponential model from the first data point, which was 1 SD above

the baseline mean through the entire response. The [HHb] TD and time constant values were summed to provide information on the overall [HHb] response dynamics in the fundamental phase of the response. The oxyhemoglobin concentration ([HbO_2]) and total hemoglobin concentration ([Hb_{tot}]) responses do not approximate an exponential (22) and were, therefore, not modeled. Rather, we assessed changes in these parameters by determining the HbO_2 and [Hb_{tot}] at baseline (90-s preceding step transition), 120 s, and end exercise (average response over the final 30 s of exercise).

We also modeled the HR response to exercise in each condition. For this analysis, HR data were linearly interpolated to provide second-by-second values, and, for each individual, identical repetitions from like transitions were time aligned to the start of exercise and ensemble averaged. Nonlinear least squares monoexponential and biexponential models without TD were used to fit the data to moderate- and severe-intensity exercise, respectively, with the fitting window commencing at $t = 0$ s. The HR τ_p so derived provides information on the overall HR response dynamics.

Statistics

Differences in the cardiorespiratory and NIRS-derived variables between conditions were analyzed with two-tailed, paired-samples *t*-tests. Alterations in BP and plasma [NO_2^-] were determined via a two-tailed, two-way (supplement $\times$ time) repeated-measures ANOVA. Significant effects were further explored using simple contrasts, with the α-level adjusted via a Bonferroni correction. Correlations were assessed via Pearson's product-moment correlation coefficient. Data are presented as means $\pm$ SD, unless otherwise stated. Statistical significance was accepted when $P < 0.05$.

RESULTS

The BR supplementation regimen implemented in this study was well tolerated with no deleterious side effects. Subjects did, however, report beeturia (red urine) and red stools, consistent with a previous study (62).

Plasma [NO_2^-] and BP

The group mean plasma [NO_2^-] values obtained at the two sample points on each of *days 4*, *5*, and *6* of the BR and PL supplementation periods are illustrated in Fig. 1. Participants showed elevations in plasma [NO_2^-] during the BR supplementation compared with PL at all sample points (Fig. 1). On average, across the six sample points, BR ingestion increased plasma [NO_2^-] by 96%. The BR-induced elevations in plasma [NO_2^-] were not different across *days 4–6*.

The group mean systolic BP values measured at the six BR and PL sample points are shown in Fig. 2. The ingestion of BR significantly reduced systolic BP at five of the six sample points, relative to PL. Overall, systolic BP was reduced by 6 mmHg across the six samples points (Fig. 2); however, similar to plasma [NO_2^-], the BR-induced reductions in systolic BP were not significantly different among *days 4–6*. The systolic BP was significantly related to the plasma [NO_2^-] on *day 5* ($r = -0.71$, $P < 0.05$), but no relationships were detected between systolic BP and plasma [NO_2^-] on *days 4* or *6*. Diastolic BP ($\sim$72 $\pm$ 8 mmHg) and mean arterial pressure ($\sim$91 $\pm$ 5 mmHg) were not significantly affected by BR ingestion.

NIRS Measurements

Moderate exercise. The [HbO_2], [HHb], and [Hb_{tot}] values measured during moderate exercise are shown in Table 1, and the group mean responses are shown in Fig. 3. A 13% reduction in the [HHb] amplitude was observed following BR ingestion, indicating that fractional O_2 extraction was reduced (PL: 88 $\pm$ 38 vs. BR: 78 $\pm$ 34 AU; $P < 0.05$; Fig. 3). The [HbO_2] within the microvasculature was increased at baseline and at 2 min into exercise, but was not significantly different at the end of exercise (Table 1). BR ingestion elevated [Hb_{tot}] (an index of vascular red blood cell content) at baseline; however, this effect was not maintained during exercise (Table 1).

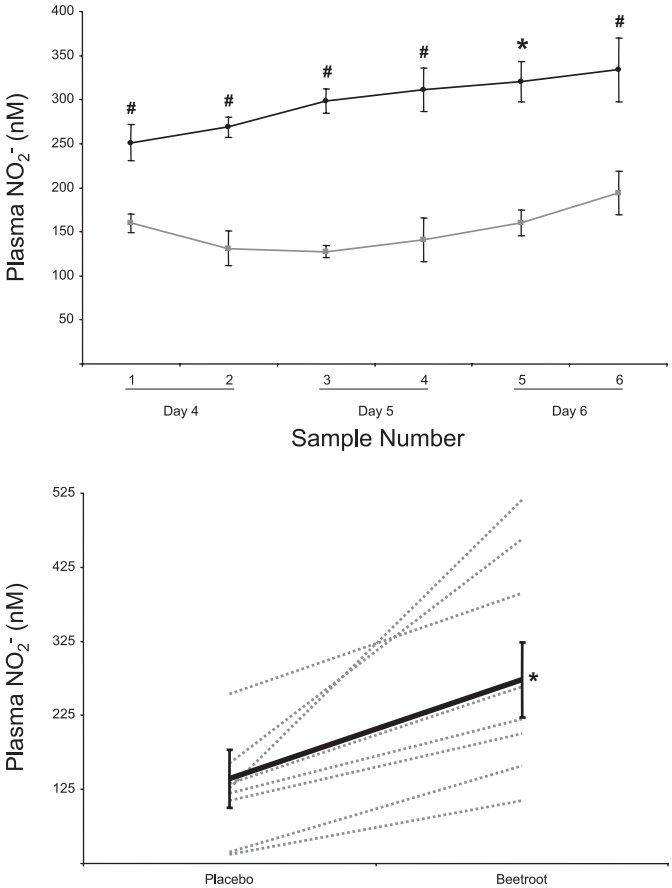

Fig. 1. Plasma nitrite concentration ([NO_2^-]) following 4–6 days of dietary nitrate or placebo supplementation. *Top*: group mean ($\pm$SE) values of plasma NO_2^- on *days 4*, *5*, and *6* of supplementation, with either nitrate (solid circles) or placebo (shaded squares). The blood samples for [NO_2^-] determination were taken before each of the six exercise bouts that were completed in each condition (*bouts 1*, *2*, *3*, and *5* were moderate, and *bouts 4* and *6* were severe; see text for further details). Note the significantly greater plasma [NO_2^-] following dietary nitrate supplementation. Significant difference from placebo at corresponding time point at the #5% and *1% levels of significance are given. *Bottom*: individual (dotted shaded lines) and mean $\pm$ SE (solid line) values for plasma [NO_2^-] measured over *days 4*, *5*, and *6*. *$P < 0.01$.

Severe exercise. The [HbO_2], [HHb], and [Hb_{tot}] values measured during severe exercise are shown in Table 1, and the group mean response is shown in Fig. 4. In contrast to the BR-induced changes in indexes of muscle oxygenation during moderate exercise, the [HHb], [HbO_2], and [Hb_{tot}] parameters were unaffected by BR ingestion during severe exercise.

$\dot{V}O_2$ Dynamics and Exercise Tolerance

Moderate exercise. The pulmonary $\dot{V}O_2$ response during moderate exercise is illustrated in Fig. 5, and the parameters derived from the model fit are presented in Table 2. Dietary BR

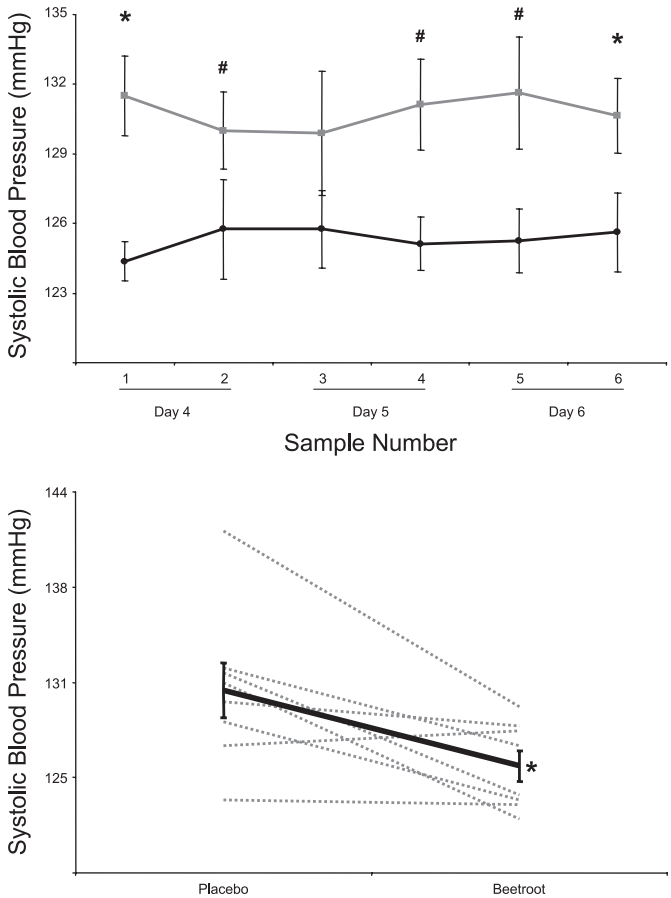

Fig. 2. Systolic blood pressure (SBP) following 4–6 days of dietary nitrate or placebo supplementation. *Top*: group mean (±SE) values of SBP on *days 4*, *5*, and *6* of supplementation with either nitrate (solid circles) or placebo (shaded squares). Note the significantly lower SBP following dietary nitrate supplementation. Significant difference from placebo at corresponding time point at the #5% and *1% levels of significance are given. *Bottom*: individual (dotted shaded lines) and mean ± SE (solid line) values for SBP measured over *days 4*, 5, and *6*. *$P < 0.01$.

supplementation resulted in a 19% reduction in the amplitude of the pulmonary $\dot{V}O_2$ response, relative to PL, following a step increment to the same absolute moderate-intensity cycling work rate (PL: 640 ± 146 vs. BR: 521 ± 153 ml/min; $P < 0.01$; Fig. 5), with there being no difference in $\dot{V}O_2$ during the baseline period of very-low-intensity (20 W) cycling. Accordingly, the functional gain (i.e., the ratio of the increase in O_2 consumed per minute to the increase of external power output) was reduced from 10.8 ml·min^{-1}·W^{-1} following PL supplementation to 8.6 ml·min^{-1}·W^{-1} following BR supplementation. The absolute $\dot{V}O_2$ value over the final 30 s of moderate exercise was also significantly lower following BR ingestion (PL: 1,517 ± 123 vs. BR: 1,448 ± 129 ml/min; $P < 0.01$; Fig. 5). The phase II time constant was not significantly altered by BR supplementation (PL: 26 ± 7 vs. BR: 29 ± 6 s; $P > 0.05$). The 95% confidence interval for the estimation of the phase II time constant was 3 ± 1 s for both conditions. The baseline and end-exercise values of CO_2 production, minute ventilation ($\dot{V}E$), respiratory exchange ratio (RER), HR, and blood [lactate] were not significantly between the conditions (Tables 2 and 3).

Severe exercise. The pulmonary $\dot{V}O_2$ response during severe exercise is shown in Fig. 6, and the parameters derived from the bi-exponential fit are presented in Table 2. In contrast to the effects observed for moderate exercise, the primary $\dot{V}O_2$ amplitude during severe exercise was significantly elevated following BR supplementation (PL: 2,158 ± 168 vs. BR: 2,345 ± 179 ml/min; $P < 0.05$). Additionally, the phase II time constant was significantly greater following BR supplementation relative to PL (PL: 33 ± 11 vs. BR: 40 ± 13 s; $P < 0.05$; Table 2). The 95% confidence intervals for the estimation of the phase II time constant were 5 ± 2 and 6 ± 3 s for the PL and BR conditions, respectively. The amplitude of the $\dot{V}O_2$ slow component was significantly smaller following BR supplementation (PL: 739 ± 242 vs. BR: 568 ± 195 ml/min; $P < 0.05$), and, therefore, represented a smaller proportion of the overall $\dot{V}O_2$ response (PL: 25 ± 6 vs. BR: 19 ± 6%; $P < 0.05$). The $\dot{V}O_2$ attained at task failure was not different, either between the conditions or from the $\dot{V}O_{2max}$ recorded during the initial ramp incremental test. Exercise tolerance was enhanced following BR supplementation, as demonstrated by the increased time to task failure (PL: 583 ± 145 vs. BR: 675 ± 203 s; $P < 0.05$; Table 2). However, the

Table 1. *Near-infrared spectroscopy-derived HHb, HbO_2, and Hb_{tot} dynamics during moderate- and severe-intensity exercise following supplementation with nitrate and placebo*

	Placebo	Nitrate
Moderate-intensity exercise		
[HHb]		
Baseline, AU	−132±84	−131±96
120 s, AU	−41±56	−54±74
End, AU	−51±55	−55±83
Mean response time, s	32±8	29±9
Amplitude, AU	88±38	78±34*
[HbO_2]		
Baseline, AU	−29±74	21±51*
120 s, AU	−80±72	−15±30*
End, AU	−5±67	25±39
[Hb_{tot}]		
Baseline, AU	−160±129	−110±89*
120 s, AU	−47±89	−29±70
End, AU	−57±92	−30±81
Severe-intensity exercise		
[HHb]		
Baseline, AU	−142±95	−104±89
120 s, AU	176±107	202±125
End, AU	215±110	246±126
Primary time constant, s	9±2	11±2
Primary time delay, s	8±1	8±2
Primary amplitude, AU	300±70	287±103
Slow-phase amplitude, AU	63±27	67±16
[HbO_2]		
Baseline, AU	96±119	57±91
120 s, AU	−147±66	−176±68
End, AU	−133±59	−166±65
[Hb_{tot}]		
Baseline, AU	−46±116	−47±69
120 s, AU	29±121	26±105
End, AU	82±110	80±104

Values are means ± SD. [HHb], deoxygenated hemoglobin concentration; [HbO_2], oxygenated hemoglobin concentration; [Hb_{tot}], total hemoglobin concentration; AU, arbitrary units. *Significantly different from placebo, $P < 0.05$.

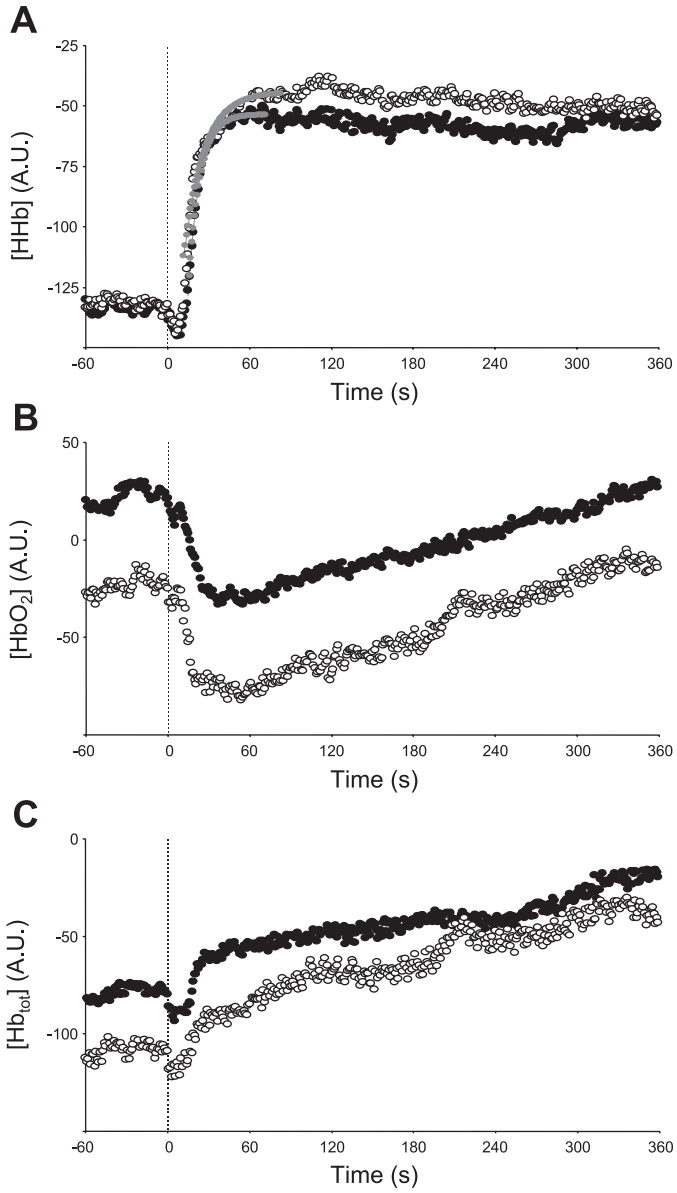

Fig. 3. Group mean changes in the parameters of muscle oxygenation following nitrate and placebo supplementation before and during a step increment to a moderate-intensity cycle work rate. Responses following nitrate supplementation are shown as solid circles, while the placebo responses are shown as open circles. The dotted vertical line represents the abrupt imposition of a moderate work rate from a baseline of "unloaded" cycling. *A*: deoxyhemoglobin concentration ([HHb]). *B*: oxyhemoglobin concentration ([HbO_2]). *C*: total hemoglobin concentration ([Hb_{tot}]). For each individual, the responses to four like-transitions were averaged together before analysis. Note the reduction in the amplitude of the [HHb] response and the greater [HbO_2] and [Hb_{tot}] before and during moderate exercise following dietary nitrate supplementation. Error bars are not shown for clarity, but see Table 1 for further details. AU, arbitrary units.

increased time to task failure was not correlated with the reduction of the $\dot{V}O_2$ slow component ($r = -0.14$; $P = 0.70$). The baseline and end-exercise values of CO_2 production, $\dot{V}E$, RER, and HR were not significantly different between the conditions (Tables 2 and 3). Blood [lactate] at 6 min of exercise and at task failure was also not significantly different between the conditions (Table 3).

DISCUSSION

The principal original finding of this investigation is that 3 days of dietary supplementation with nitrate-rich BR (which doubled the plasma [nitrite]) significantly reduced the O_2 cost of cycling at a fixed submaximal work rate and increased the time to task failure during severe exercise. These findings were consistent with our experimental hypotheses. The O_2 cost of cycling at a fixed moderate work rate is known to be highly consistent in human populations, irrespective of factors such as age and training status (36, 49). That an acute nutritional

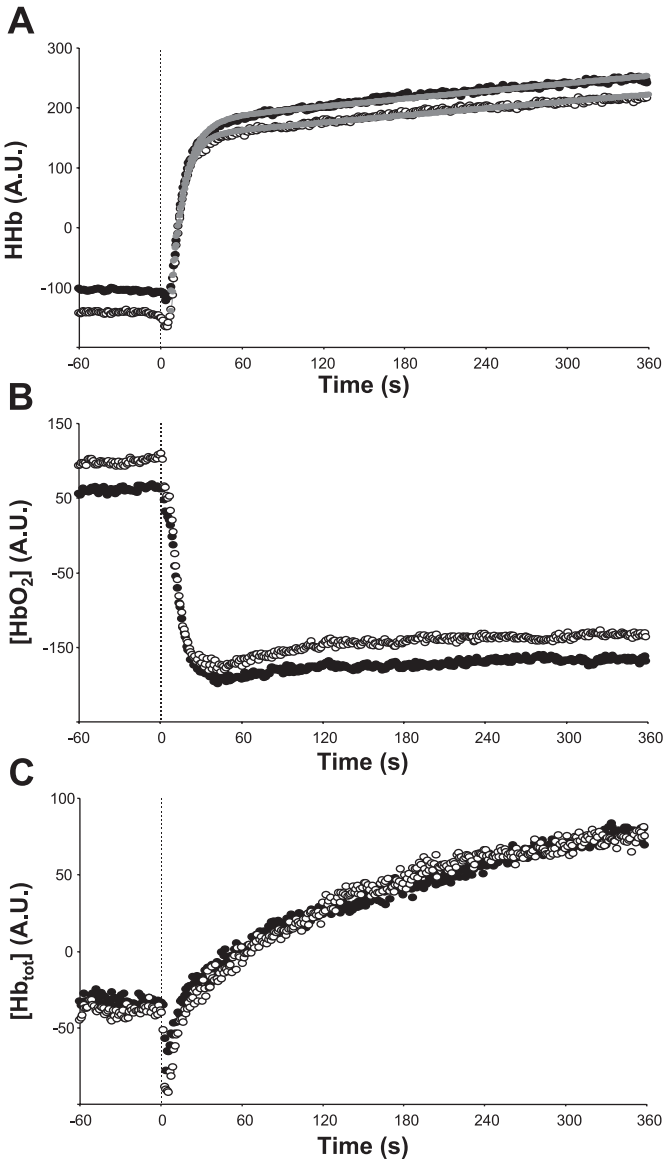

Fig. 4. Group mean changes in the parameters of muscle oxygenation following nitrate and placebo supplementation before and during a step increment to a severe-intensity cycle work rate. Responses following nitrate supplementation are shown as solid circles, while the placebo responses are shown as open circles. The dotted vertical line represents the abrupt imposition of a severe work rate from a baseline of "unloaded" cycling. *A*: [HHb]. *B*: [HbO_2]. *C*: [Hb_{tot}]. For each individual, the responses to two like-transitions were averaged together before analysis. The near-infrared spectroscopy-derived parameters were not appreciably different before or during severe exercise following dietary nitrate supplementation. Error bars are not shown for clarity, but see Table 1 for further details.

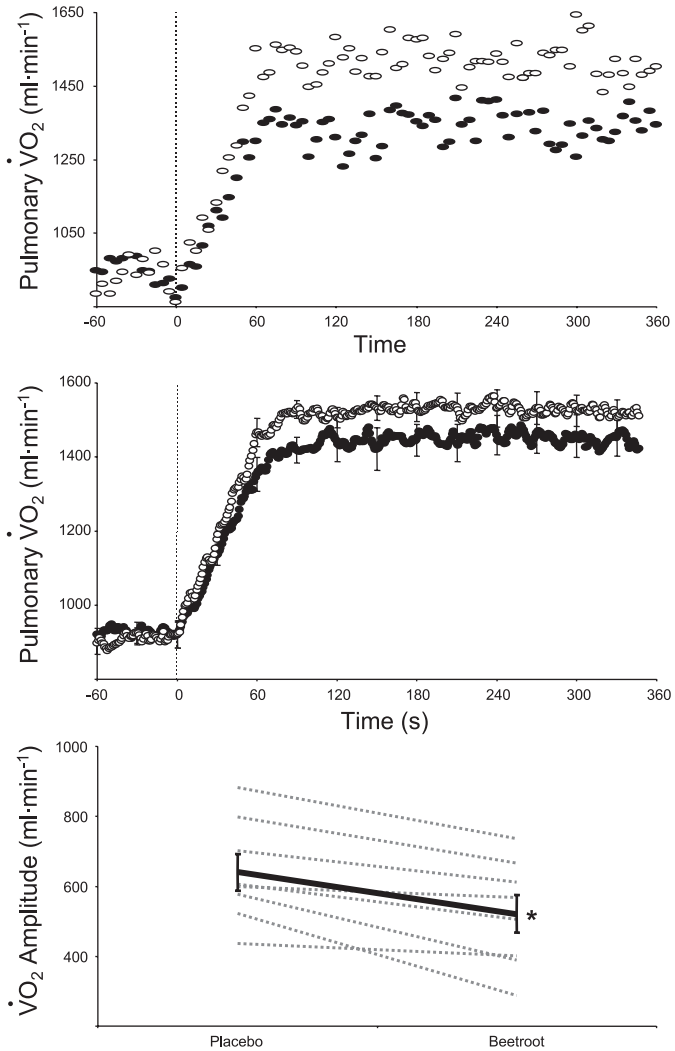

Fig. 5. Pulmonary oxygen uptake ($\dot{V}O_2$) response following nitrate and placebo supplementation during a step increment to a moderate-intensity work rate. Responses following nitrate supplementation are shown as solid circles, while the placebo responses are shown as open circles. The dotted vertical line represents the abrupt imposition of the moderate work rate from a baseline of "unloaded" cycling. *Top*: $\dot{V}O_2$ response of a representative individual (data are shown at 5-s intervals). *Middle*: group mean $\dot{V}O_2$ response with error bars shown every 30 s for clarity. The oxygen cost of moderate exercise was significantly reduced following beetroot supplementation. *Bottom*: individual changes in the amplitude of the $\dot{V}O_2$ response to moderate exercise following nitrate supplementation (dotted shaded lines), along with the group mean change (solid line). For each individual, the responses to four like-transitions were averaged together before analysis. Note that the effect was observed in all participants. *$P < 0.01$.

intervention (i.e., dietary supplementation with a natural food product that is rich in nitrate) can reduce the O_2 cost of a given increment in work rate by ~20% is, therefore, remarkable.

Short-term (i.e., 4–6 days) dietary supplementation with BR increased plasma [NO_2^-] by ~96% in this investigation. Consistent with these findings, dietary NO_3^- supplementation has previously been shown to increase plasma [NO_2^-] when administered as either sodium nitrate (45) or BR (62). Importantly, interrupting the entero-salivary circulation by spitting out saliva thwarted the rise in plasma [NO_2^-] (62), while administration of antibacterial mouthwash before sodium nitrate ingestion also prevented the rise in plasma [NO_2^-] by decreasing the NO_3^--reducing bacteria counts in the oral cavity (28). Collectively, these data highlight the dependence of the NO_3^--to-NO_2^- conversion pathway on the commensal bacterial nitrate reductases present in the human oral cavity. The bacterially derived NO_2^- can increase circulating plasma NO_2^- and undergo reduction to yield NO in hypoxia or acidosis (21, 46).

Effects of dietary nitrate on BP. In the present study, dietary supplementation with BR reduced systolic BP by an average of 6 mmHg, but without altering diastolic BP or mean arterial pressure. In contrast, both systolic and diastolic BP were reduced following 3 days of dietary NO_3^- supplementation in the study of Larsen et al. (45). Recent data indicate that, following BR ingestion, peak reductions in systolic and diastolic BP are observed 2.5 and 3 h postingestion, respectively (62). Furthermore, the BR-induced reduction in systolic BP persisted for 24 h postingestion, while diastolic BP had returned toward baseline (62). Collectively, these data suggest that systolic BP is more amenable to nitrate-induced change than is diastolic BP. The reduced BP observed with a diet rich in nitrates

Table 2. *Mean ± SD ventilatory and gas exchange dynamics during moderate- and severe-intensity exercise following supplementation with nitrate and placebo*

	Placebo	Nitrate
Moderate-intensity exercise		
$\dot{V}O_2$		
Baseline, l/min	0.91±0.09	0.93±0.05
End-exercise, l/min	1.52±0.12	1.45±0.13*
Phase II time constant, s	26±7	29±6
Mean response time, s	39±8	45±4
Primary amplitude, l/min	0.64±0.15	0.52±0.15*
Primary gain, ml·min^{-1}·W^{-1}	10.8±1.6	8.6±0.7†
$\dot{V}CO_2$		
Baseline, l/min	0.85±0.08	0.84±0.05
End-exercise, l/min	1.31±0.15	1.32±0.15
$\dot{V}E$		
Baseline, l/min	25±2	24±1
End-exercise, l/min	36±4	34±3
Respiratory exchange ratio		
Baseline	0.93±0.07	0.90±0.07
End-exercise	0.90±0.05	0.91±0.02
Severe-intensity exercise		
$\dot{V}O_2$		
Baseline, l/min	0.99±0.10	0.96±0.07
End-exercise, l/min	3.87±0.29	3.82±0.28
Phase II time constant, s	33±11	40±13†
Primary amplitude, l/min	2.19±0.17	2.35±0.18†
Primary gain, ml·min^{-1}·W^{-1}	9.0±0.7	9.4±0.6
Slow-phase amplitude, l/min	0.74±0.24	0.57±0.20†
Slow-component amplitude, %	25±6	19±6†
Overall gain, ml·min^{-1}·W^{-1}	11.6±0.9	10.8±0.8*
Overall mean response time, s	75±16	71±16
$\dot{V}CO_2$		
Baseline, l/min	0.85±0.23	0.86±0.10
End-exercise, l/min	3.99±0.32	4.03±0.43
$\dot{V}E$		
Baseline, l/min	24±7	25±3
End-exercise, l/min	140±14	139±21
Respiratory exchange ratio		
Baseline	0.86±0.17	0.89±0.09
End-exercise	1.04±0.05	1.05±0.05

Values are means ± SD. $\dot{V}O_2$, oxygen uptake; $\dot{V}CO_2$, expired carbon dioxide; $\dot{V}E$, minute ventilation. Significantly different from placebo: *$P < 0.01$, †$P < 0.05$.

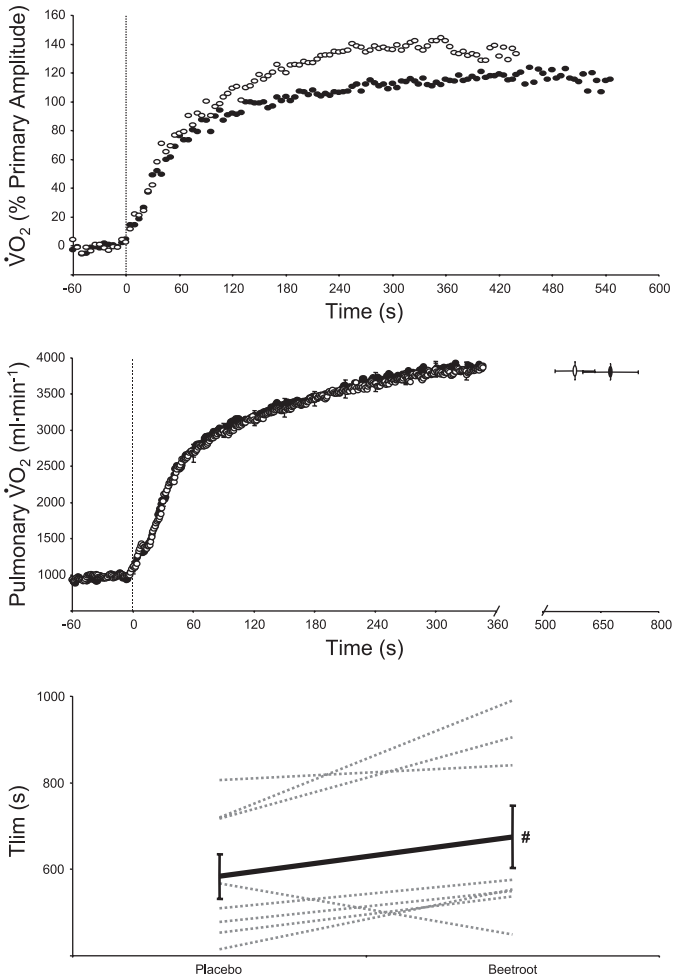

Fig. 6. Pulmonary $\dot{V}O_2$ response following nitrate and placebo supplementation during a step increment to a severe-intensity work rate. Responses following nitrate supplementation are shown as solid circles, while the placebo responses are shown as open circles. The dotted vertical line represents the abrupt imposition of the severe work rate from a baseline of "unloaded" cycling. *Top*: $\dot{V}O_2$ response of a representative individual (data are shown at 5-s intervals). The data are plotted as a fraction of the $\dot{V}O_2$ fundamental component amplitude to more clearly illustrate the slower phase II $\dot{V}O_2$ kinetics and reduced $\dot{V}O_2$ slow component following nitrate supplementation. *Middle*: group mean $\dot{V}O_2$ response with error bars shown every 30 s for clarity. The group mean ± SE $\dot{V}O_2$ at task failure is also shown. *Bottom*: individual changes in the tolerance of severe exercise following nitrate supplementation (dotted shaded lines) along with the group mean change (solid line). #$P <$ 0.05.

suggests that this "natural" approach has the potential to maintain or enhance aspects of human cardiovascular health.

The NO_3^--induced reduction in systolic BP is likely mediated via its conversion to NO_2^- and thence NO. NO is known to be an important endothelial relaxing factor, through its role as a secondary messenger in cyclic guanosine monophosphate synthesis, culminating in smooth muscle relaxation (31). Conventionally, NO production was considered to be derived, universally, via the NO synthase family of enzymes in humans. These enzymes produce NO through catalyzing the five-electron oxidation of L-arginine in a reaction requiring O_2 and NADPH (9). More recent research has identified an O_2-independent pathway for the generation of NO, via the reduction of NO_2^- to NO in an acidic milieu (6). Importantly, this pathway preserves blood and tissue NO production during hypoxia when the enzymatic activity of the NO synthases are rate limited by the lack of O_2 availability. As such, a considerable body of evidence now supports a biological role for NO_2^- in hypoxic vasodilation (26), which serves to protect tissues from ischemia and reperfusion injury (27).

Effects of dietary nitrate on the physiological responses to moderate exercise. Increased dietary NO_3^- consumption altered indexes of muscle oxygenation as investigated using NIRS. During moderate exercise, [Hb_{tot}] was elevated at baseline, and [HbO_2] was elevated both at baseline and over the first 120 s of exercise following BR ingestion. The increased blood volume in the region of interrogation at baseline following BR ingestion is presumably a consequence of enhanced muscle vasodilatation, resulting from increased NO production from NO_2^-. The NIRS-derived [HHb] response reflects the balance between local O_2 delivery and utilization and has been used previously as an index of muscle fractional O_2 extraction (1, 22, 24, 30). In the present study, the amplitude of the [HHb] response was reduced by 13% following BR ingestion. Conversely, inhibition of NO synthesis via N^G-nitro-L-arginine methyl ester (L-NAME) administration has been shown to increase muscle O_2 extraction in the exercising horse (40). By the Fick equation, for the same $\dot{V}O_2$, an increased muscle O_2 delivery would be expected to enable a reduced muscle fractional O_2 extraction. However, in the present study, in which $\dot{V}O_2$ was reduced by dietary BR supplementation, an alternative interpretation is that less O_2 extraction was required consequent to a reduced aerobic energy turnover or muscle energy utilization.

Perhaps the most striking finding of the present investigation was the significant reduction in the O_2 cost of submaximal exercise following increased dietary NO_3^- intake. While the $\dot{V}O_2$ response during the unloaded baseline cycling period was unaffected, a 19% reduction in the amplitude of the pulmonary

Table 3. *Heart rate and blood lactate responses to moderate- and severe-intensity exercise following supplementation with nitrate and placebo*

	Placebo	Nitrate
Moderate-intensity exercise		
Heart rate		
Baseline, beats/min	81±9	81±8
End, beats/min	98±12	98±13
Time constant, s	28±12	31±16
Blood [lactate]		
Baseline, mM	1.0±0.5	0.9±0.3
End, mM	1.2±0.7	1.1±0.2
Δ, mM	0.2±0.2	0.2±0.2
Severe-intensity exercise		
Heart rate		
Baseline, beats/min	85±8	88±8
End, beats/min	170±8	170±8
Time constant, s	16±10	17±5
Blood [lactate]		
Baseline, mM	1.0±0.2	1.1±0.5
End, mM	6.9±1.6	6.9±1.2
Δ, mM	5.9±1.6	5.7±1.0
Exhaustion, mM	10.0±1.9	10.0±1.7

Values are means ± SD. [Lactate], lactate concentration; Δ, change.

$\dot{V}O_2$ response, relative to PL, was evident following NO_3^- supplementation during a step increment to the same absolute moderate-intensity cycling work rate. Accordingly, the functional gain (i.e., the ratio of the increase in O_2 consumed per minute to the increase of external power output and the reciprocal of delta efficiency; Ref. 64) was reduced from 10.8 $ml \cdot min^{-1} \cdot W^{-1}$ following PL supplementation to 8.6 $ml \cdot min^{-1} \cdot W^{-1}$ following NO_3^- supplementation. Moreover, the gross O_2 cost of exercise (comprising resting metabolic rate, the O_2 cost of moving the limbs during baseline pedaling, and the O_2 cost of muscle contraction to meet the imposed work rate) was reduced by ~5%. The magnitude of effect was similar to that reported by Larsen et al. (45) using sodium nitrate supplementation. While we therefore suggest that the reduced O_2 cost of submaximal exercise in our study was consequent to the increased dietary nitrate content, we cannot presently exclude the possibility that other substances contained in BR contributed to the results we obtained. Importantly, the reduction in $\dot{V}O_2$, and thus of ATP resynthesis through oxidative phosphorylation, was not compensated by elevations in glycolytic ATP provision, as inferred, albeit crudely, from the similar blood [lactate] values between the BR and PL conditions. These findings extend those of Larsen et al. (45) by demonstrating that the O_2 cost of submaximal exercise is reduced following NO_3^- ingestion in the form of a natural food product. That HR and $\dot{V}E$ were not significantly different between treatments suggests that the reduction in $\dot{V}O_2$ originated from the skeletal muscles and not from alterations in the energetic cost of cardiorespiratory support processes. Moreover, the similar RER between the conditions indicates that substrate utilization (which can influence the O_2 cost of exercise) was not altered by the intervention.

The mechanistic bases for the reduced O_2 cost of submaximal exercise following increased NO_3^- intake, either by pharmacological (45) or natural dietary means (present study), are unclear. The inhibition of NO synthesis has previously been shown to increase steady-state $\dot{V}O_2$ in dogs (56), but not humans (38) or horses (41). It is widely accepted, however, that NO is involved in the regulation of mitochondrial O_2 consumption. In particular, it has been established that NO has a strong affinity for cytochrome-*c* oxidase (CytOX; Ref. 10), but there is also evidence that NO has the potential to modulate other aspects of mitochondrial and muscle contractile function (11, 57, 58).

A reduction in the O_2 cost of mitochondrial ATP resynthesis would require either more protons pumped per O_2 molecule reduced, or the use of an alternative terminal electron acceptor in place of O_2. It has been proposed that mitochondrial efficiency is intimately linked to the process of uncoupled respiration in which mitochondrial proton leak results in energy dissipation as heat instead of conversion to ATP (8). In this regard, the improved O_2 efficiency noted in the present study following BR ingestion might be related to a reduction of mitochondrial proton leak or proton pump slippage. There is evidence to suggest that NO increases the efficiency of oxidative phosphorylation in isolated mitochondria by reducing slipping of the proton pumps (17). Another possibility is that NO_2^- could be acting in place of O_2 as the final electron acceptor in the respiratory chain, thereby reducing the requirement for O_2 consumption (3). An intra-mitochondrial NO_2^- regeneration pathway would be critical in this scenario, given the limited NO_2^- concentration within the mitochondria. Under conditions of low electron flux, NO can inhibit CytOX by binding to the Cu^{2+} active site yielding nitrosonium (NO^+), which is subsequently hydrated to NO_2^- (18). One possible scenario is that the NO_2^- so produced could be reduced to NO by accepting an electron from CytOX, and this NO could subsequently bind to the Cu^{2+} active site, completing the cyclical process to regenerate NO_2^-. This possibility is intriguing, as the hydration of NO^+ to NO_2^- yields an electron that can be redistributed within CytOX (19, 60). Subsequently, this electron may be accepted by NO_2^-, potentially utilizing the electron derived from its synthesis, and could contribute to proton pumping and ATP synthesis, in an efficiently coupled process.

The reduction in O_2 consumption with increased dietary NO_3^- intake could also be attributed, in part, to a reduced ATP cost of force production, requiring less flux through oxidative phosphorylation. One of the most energetically costly processes during skeletal muscle contraction is sarcoplasmic reticulum Ca^{2+} pumping, which may account for up to 50% of the total ATP turnover (7). The presence of reactive oxygen species increases the opening probability of the sarcoplasmic reticulum Ca^{2+} release channels (48), and the active reuptake of the elevated cytosolic Ca^{2+} would present a considerable energetic challenge (7). NO donors that invoke small elevations in NO might protect the channel against oxidation-induced Ca^{2+} release, without significantly altering channel function (53). Therefore, the BR-induced elevations in NO may have prevented an excess of Ca^{2+} release and subsequently reduced the considerable energetic cost of its resequestration. These suggestions are naturally speculative at the present time and await further investigation.

Effects of dietary nitrate on the physiological responses to severe exercise. The physiological responses during severe exercise were different from those observed during moderate exercise following BR supplementation, suggesting that the influence of NO_2^- and/or NO on muscle function is specific to the exercise intensity domain being investigated. First, there were no significant differences in NIRS-derived indexes of muscle oxygenation between the NO_3^- and PL conditions during severe exercise, although this might be a function of the fact that severe exercise always followed moderate exercise in our experiments. Moreover, in contrast to the reduced steady-state $\dot{V}O_2$ observed during moderate exercise following increased NO_3^- consumption, the amplitude of the primary or fundamental component $\dot{V}O_2$ response during severe exercise was increased (by ~7%), and the amplitude of the subsequent $\dot{V}O_2$ slow component was reduced (by ~23%), with the $\dot{V}O_2$ at the point of task failure being not significantly different between the conditions. These changes in $\dot{V}O_2$ kinetics during high-intensity exercise following dietary NO_3^- supplementation resemble those that are observed following an initial "priming" bout of high-intensity exercise (15, 35, 45), effects that have been attributed, either separately or in combination, to increased muscle O_2 delivery, increased oxidative metabolic enzyme activity and carbon substrate availability, and altered motor unit recruitment patterns (15, 35, 45). We have previously reported that NO synthase inhibition with L-NAME significantly increased the amplitude of the $\dot{V}O_2$ slow component and speculated that this might be related to changes in muscle O_2 delivery or its distribution and/or to (related)

changes in motor unit recruitment patterns (39). It is presently unclear which, if any, of the above-named mechanisms contributed to the altered $\dot{V}O_2$ kinetics during severe exercise following dietary NO_3^- supplementation. Myocytes in close proximity to a capillary are advantaged with respect to muscle O_2 availability, whereas myocytes situated further away are increasingly less well supplied with O_2, creating an O_2 pressure gradient within the contracting muscles. The hypoxic and acidic milieu within and surrounding the distal myocytes might stimulate NO_2^- reduction to NO facilitating vasodilatation and thus delivery of O_2. The NO so produced is capable of diffusion and may inhibit mitochondrial O_2 consumption in the myocytes proximal to the capillary bed, promoting deeper diffusion of the available O_2 (32, 59) and, therefore, enabling a more appropriate matching of local O_2 delivery to O_2 requirement. Another possibility is that the increased NO availability following increased dietary NO_3^- intake promotes mitochondrial biogenesis (16, 50). Greater mitochondrial volume would enable the same rate of mitochondrial respiration to be accomplished with a reduced perturbation of adenine nucleotides. However, irrespective of the mechanism(s) involved, the return of $\dot{V}O_2$ kinetics toward first-order linear system dynamics during severe exercise will likely limit the rate at which metabolites that have been associated with the fatigue process (e.g., H^+, P_i, ADP) accumulate in skeletal muscle (14), effects that would be expected, in turn, to portend enhanced exercise tolerance.

The τ_p was significantly longer (i.e., phase II pulmonary $\dot{V}O_2$ kinetics was slower) following NO_3^- supplementation relative to PL for severe exercise (group mean τ_p 40 vs. 33 s for BR and PL). This is consistent with our laboratory's previous reports that the τ_p was significantly faster when NOS activity was inhibited by L-NAME (38, 39, 66). The τ_p was also slightly longer during moderate exercise (group mean τ_p 29 vs. 26 s for BR and PL), but this difference was not statistically significant. While it is acknowledged that changes in muscle blood flow, which might occur with greater or lesser NO availability, have the potential to dissociate the normally close relationship between muscle and pulmonary $\dot{V}O_2$ kinetics (2), our data suggest that NO might have an important regulatory influence on the inertia of $\dot{V}O_2$ dynamics following the onset of exercise (36, 51). It is of interest that NO_3^- supplementation resulted in changes in the amplitudes of the $\dot{V}O_2$ response to exercise (i.e., lower steady-state $\dot{V}O_2$ during moderate exercise and higher $\dot{V}O_2$ fundamental component and reduced $\dot{V}O_2$ slow component during severe exercise), while also slowing the phase II $\dot{V}O_2$ kinetics. While the mechanistic bases of this effect is not clear, it is possible that increased NO availability results, simultaneously, in a slowing of the rate at which $\dot{V}O_2$ rises following the onset of exercise (via competitive inhibition of CytOX; Refs. 10, 38, 39, 41, 56) and changes in the amplitudes of the fundamental and slow components of $\dot{V}O_2$ (through effects on the efficiency of muscle oxidative metabolism and/or contractile function; Refs. 3, 11, 17, 57).

The kinetics of $\dot{V}O_2$ are considered to be an important determinant of exercise tolerance (14, 36). However, in this study, we observed a 16% improvement in the time to task failure during severe exercise in the NO_3^- condition, despite the slower phase II $\dot{V}O_2$ kinetics. Another parameter of $\dot{V}O_2$ dynamics considered to influence exercise tolerance is the slow component rise in $\dot{V}O_2$ observed during supra-GET exercise, since this parameter is associated with greater utilization of the finite phosphocreatine (55) and glycogen (42) reserves. Indeed, a reduction in the $\dot{V}O_2$ slow-component amplitude has been associated with improved exercise tolerance (1, 14). However, in the present study, the improvement in severe exercise tolerance with NO_3^- supplementation was not significantly correlated with the reduction of the $\dot{V}O_2$ slow-component amplitude. The $\dot{V}O_2$ at task failure (which was equivalent to the preestablished $\dot{V}O_{2max}$) was not different between conditions, suggesting that the $\dot{V}O_{2max}$ was reached more slowly and/or could be sustained for longer following NO_3^- supplementation. It is noteworthy that the $\dot{V}O_{2max}$ during severe exercise was not impaired with NO_3^- supplementation, although the steady-state $\dot{V}O_2$ was reduced during submaximal exercise. These results differ from those obtained with NO synthase inhibition. With the latter, $\dot{V}O_{2max}$ and exercise tolerance are impaired during both ramp incremental exercise (37) and supramaximal step exercise (66). Although the mechanism for the enhanced performance observed in the present study is uncertain, an interesting possibility is that an elevation of tissue s-nitrosothiols with increased dietary nitrate intake (13) prevents those nitrosylated thiols undergoing irreversible oxidative modification as a consequence of the production of reactive oxygen species (12, 54), an effect that is known to compromise skeletal muscle force production (25). We wish to stress here that, while the 16% improvement in the time to task failure during severe constant work rate exercise is impressive, the magnitude of effect would be expected to be much smaller during time trial exercise tasks in which a given distance is completed in the shortest possible time (34). Nevertheless, it is possible that the effect might still be meaningful in terms of performance enhancement.

Applications and conclusions. A short period of dietary NO_3^- supplementation through a natural food product resulted in increased plasma [nitrite] and reduced systolic BP in the normotensive young adult men who participated in our study. During exercise at a fixed moderate work rate, increased NO_3^- intake resulted in improvements in NIRS-derived indexes of muscle oxygenation and a significant reduction in pulmonary $\dot{V}O_2$. It should be stressed that the remarkable reduction in the O_2 cost of submaximal cycle exercise following dietary supplementation with inorganic nitrate in the form of a natural food product cannot be achieved by any other known means, including long-term endurance exercise training (1, 15, 49, 65). Although not directly tested in the present study, the results suggest that increased dietary NO_3^- intake has the potential to enhance exercise tolerance during longer term endurance exercise. Moreover, in certain human populations (including the senescent and those with cardiovascular, respiratory, or metabolic diseases), the activities of daily living are physically difficult because they have an energy requirement that represents a high fraction of the $\dot{V}O_{2max}$. A reduction in the $\dot{V}O_2$ associated with such activities following dietary nitrate supplementation, therefore, has the potential to improve exercise tolerance and the quality of life in these groups. During exercise at a fixed severe work rate, BR ingestion reduced the amplitude of the $\dot{V}O_2$ slow component and increased the time to task failure by ~16%, suggesting that dietary nitrate supplementation might enhance high-intensity exercise performance. Further research is required to investigate the mechanistic bases for the reduced O_2 cost of submaximal exercise

observed with increased dietary nitrate intake in this study and previously (Ref. 45; i.e., reduced ATP cost of force production and/or increased mitochondrial P/O ratio; i.e., ratio of phosphate radicals esterified to atoms of oxygen consumed). Finally, the possible ergogenicity of dietary nitrate supplementation during different types of exercise in humans is likely to be a fertile area for further research.

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