Beetroot Juice Supplementation Does Not Improve Performance of Elite 1500-m Runners

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ABSTRACT

BOORSMA, R. K., J. WHITFIELD, and L. L. SPRIET. Beetroot Juice Supplementation Does Not Improve Performance of Elite 1500-m Runners. *Med. Sci. Sports Exerc.*, Vol. 46, No. 12, pp. 2326–2334, 2014. **Purpose**: Dietary nitrate supplementation with beetroot juice (BR) has received widespread attention as an ergogenic aid. However, recent evidence in well-trained cyclists has not consistently reported improved cycling economy or performance. The present study examined the effects of acute and chronic BR supplementation on $\dot{V}O_2$ during submaximal running and 1500-m time trial (TT) performance of elite distance runners. **Methods**: Eight male 1500-m runners ($\dot{V}O_{2peak}$, 80 ± 5 mL·kg^{-1}·min^{-1}; 1500-m personal best, 3:56 ± 9 s) participated in this study. In a randomized, double-blind, crossover design, subjects supplemented with BR or a nitrate-free BR placebo (PL) for 8 d separated by at least 1 wk. On days 1 (acute) and 8 (chronic), subjects ingested 210 mL of BR (19.5-mmol nitrate) or PL and completed a submaximal treadmill run and 1500-m TT on an indoor 200-m track. **Results**: Plasma nitrate increased from 37 ± 15 to 615 ± 151 μM (acute) and 870 ± 259 μM (chronic) after BR supplementation. There were no $\dot{V}O_2$ differences between conditions at 50%, 65%, and 80% $\dot{V}O_{2peak}$ (acute PL, 4194 ± 90 mL·min^{-1}; chronic PL, 4216 ± 95 mL·min^{-1}; acute BR, 4192 ± 113 mL·min^{-1}; chronic BR, 4299 ± 92 mL·min^{-1}). The 1500-m TT was unaffected by acute or chronic BR supplementation (acute PL, 4:10.4 min:s ± 2.5 s; chronic PL, 4:11.4 min:s ± 2.7 s; acute BR, 4:10.7 min:s ± 1.5 s; chronic BR, 4:10.5 min:s ± 2.2 s). However, two subjects improved their TT performance after acute (5.8 and 5.0 s) and chronic BR supplementation (7.0 and 0.5 s). **Conclusions**: Acute and chronic BR supplementation did not reduce running $\dot{V}O_2$ or improve 1500-m TT performance of a group of elite distance runners, but two responders to BR were identified. **Key Words:** NITRIC OXIDE, NITRITE, NITRATE, 1500-M PERFORMANCE, EXERCISE ECONOMY

Nitric oxide (NO) is a potent signaling molecule that affects cellular function in many tissues of the body. NO is produced endogenously by NO synthases (NOS) from the oxidation of L-arginine (27). In addition, nitrate (NO_3^-) supplementation, via beetroot juice (BR) or nitrate salts, has recently gained considerable attention as a dietary means to increase NO bioavailability (1,20,33). Dietary sources of nitrate are absorbed into the circulation and are taken up by the salivary glands and concentrated in the saliva (25). In the mouth, commensal anaerobic bacteria reduce nitrate to nitrite (NO_2^-). Most swallowed nitrite is reduced to NO in the stomach, but some enters the systemic circulation. At the muscular level, NO_2^- reduction to NO is facilitated by hypoxia and low pH (26). In this way, nitrite maintains NO bioavailability in hypoxic and acidic conditions that may be present during exercise. Thus, NO_2^- reduction to NO represents an alternative pathway for the generation of NO that complements the classical NOS-derived production (3).

Remarkably, nitrate supplementation has been shown to improve whole-body exercise economy (1,2,8,24,30,33). Supplementation with pharmacological sodium nitrate or nitrate-rich BR has consistently reduced steady-state $\dot{V}O_2$ by 3%–14% in recreationally active men during constant work rate cycling (1,22–24,30,33), walking and running (20), and knee extensor exercise (2). Decreased $\dot{V}O_2$ at a fixed external power output after nitrate supplementation could result from a decreased energy cost of muscle force production and/or greater adenosine triphosphate production per unit oxygen consumed (2,24). In addition to improved exercise economy, nitrate supplementation enhanced exercise tolerance by 3%–25% in cycling and running time-to-exhaustion tests in recreationally active men (1,19,20,33) and improved performance by 1.2%–2.8% during cycling time trial (TT) tests in moderately trained individuals (8,21).

Despite the considerable evidence of the positive ergogenic effects of nitrate supplementation in recreationally active and moderately trained individuals, recent studies have failed to show improved exercise economy or performance of well-trained individuals (5,10,28,32). This conclusion was supported by a recent meta-analysis where the authors suggested that more data are required to clarify the effects of BR supplementation in athletic populations (14). In these five studies on well-trained subjects, where the

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Submitted for publication November 2013.
Accepted for publication March 2014.

0195-9131/14/4612-2326/0
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DOI: 10.1249/MSS.0000000000000364

$\dot{V}O_{2peak}$ of the subjects was ≥60 mL·kg^{-1}·min^{-1} and subjects trained for competition rather than recreation, none have reported a significant improvement in mean TT performance after nitrate supplementation. In addition, these studies observed no change in exercise economy (5,10,28) or a diminished improvement (≤5%) (4,32) compared with that seen in moderately trained subjects (7%–11%) (21).

The reason for the lack of effect observed in well-trained individuals is not clearly understood. It is possible that nitrate supplementation fails to elicit a response in well-trained subjects because supplementation does not augment the contribution of endogenous NO_2^- to NO production during exercise. There is evidence showing that exercise training increases endothelial NOS expression and activity, leading to increased plasma [NO_3^-] and [NO_2^-] (13). Increased plasma [NO_2^-] may provide well-trained individuals sufficient endogenous nitrite to meet their demands. In addition, increased skeletal muscle capillary density, observed in well-trained individuals (7), may reduce the development of hypoxic loci within the skeletal muscle and decrease reliance on the production of NO from nitrite during exercise (32). If this is the case, there may be a threshold where further increases in NO_2^- via dietary nitrate supplementation will not confer additional improvements.

However, the current literature cannot rule out the possibility that well-trained subjects require greater nitrate exposure via a higher dose and/or longer periods of supplementation to elicit an improvement in exercise economy and performance. There is evidence that shows that recreationally active subjects who had no reduction in submaximal exercise $\dot{V}O_2$ at a low dose (4.2-mmol NO_3^-) responded positively to nitrate supplementation at higher doses (8.4- and 16.8-mmol NO_3^-) (33). However, the dose–response relation has not been investigated in well-trained subjects and these athletes may respond positively to nitrate supplementation if the nitrate dose was increased above the 5- to 11-mmol NO_3^- administered in previous studies. Furthermore, in well-trained subjects, a positive response may be elicited if a longer duration of supplementation is administered (9). However, the evidence to support this is equivocal and confounded by differences in training status and the different lengths of performance TT used between studies (8,9,21,32). In any case, some underlying mechanisms shown to enhance exercise economy and performance of recreationally active subjects will require time for protein modifications (i.e., citrate synthase, electron chain proteins, uncoupling protein 3, and adenine nucleotide translocase). Logically, an extended supplementation protocol would be more likely to produce these changes than an acute dose, given only 2.5 h before exercise.

Nitrate supplementation, in the form of nitrate-rich BR, before competition has become increasingly popular among athletes and could represent a legal and healthy way to improve performance of elite athletes. Therefore, the purpose of the current study was to determine the effects of acute and chronic BR supplementation on oxygen uptake during submaximal treadmill running and 1500-m track TT performance of elite middle distance runners. We aimed to determine whether nitrate supplementation could improve economy and performance if the nitrate dose and duration of supplementation were increased above those previously tested. We hypothesized that plasma [NO_3^-] would be significantly increased compared with basal concentrations after supplementation with concentrated nitrate-rich BR (19.5-mmol NO_3^-) in elite 1500-m runners. However, on the basis of recent findings, we hypothesized that neither acute nor chronic BR supplementation would reduce $\dot{V}O_2$ during submaximal treadmill running or improve 1500-m TT performance.

METHODS

Subjects. Ten elite male distance runners were recruited to participate in this study. One subject withdrew from the study after completing only one of four experimental trials because of injury. One other subject could only complete two of four experimental trials because of training and competition conflicts. Therefore, all data and analyses presented are for the eight subjects who completed all four experimental trials (mean ± SD: age, 23.8 ± 5 yr; weight, 65.7 ± 7 kg; $\dot{V}O_{2peak}$, 80 ± 5 mL·kg^{-1}·min^{-1}; 1500-m personal best, 3:56 min:s ± 9 s). Subjects were all club athletes competing in provincial-, national-, or international-caliber events and had experience competing in the 1500-m distance. Subjects completed 12.3 ± 4 h of training each week. All subjects were given an explanation of the requirements and potential risks of the study, and a written informed consent was obtained. The study was approved by the research ethics board of the University of Guelph.

Preexperimental tests. Initially, each subject completed an incremental running test to exhaustion on a treadmill (Livestrong LS13.0T; Johnson Health Tech) for determination of $\dot{V}O_{2peak}$ and the running speed required to elicit 50%, 65%, and 80% of $\dot{V}O_{2peak}$. Subjects began running at 14.3 km·h^{-1} and an incline of 0%, and the speed and incline were increased incrementally until voluntary exhaustion. Ventilation and expired oxygen and carbon dioxide concentrations were measured (MOXUS Modular $\dot{V}O_2$ System; AEI Technologies, Pittsburgh, PA) for the duration of the treadmill run. $\dot{V}O_{2peak}$ was determined as the greatest $\dot{V}O_2$ averaged over 40 s. For familiarization, subjects completed a practice exercise test consisting of a complete submaximal treadmill run and 1500-m TT (described in detail in the next section).

Study design. In a randomized, double-blind, crossover design, subjects supplemented with concentrated BR (6.5-mmol NO_3^-/70-mL Beet It Sport; James White Drinks, Ipswich, United Kingdom) or a nitrate-free BR placebo (PL) (0.065-mmol NO_3^-/70-mL Beet It Sport) for 8 d separated by 4 ± 4 wk (Fig. 1). The PL drink was created by passing the juice through an ion exchange resin that selectively removed the nitrate ions (21). The BR and PL drinks were supplied in identical packaging and were indistinguishable by

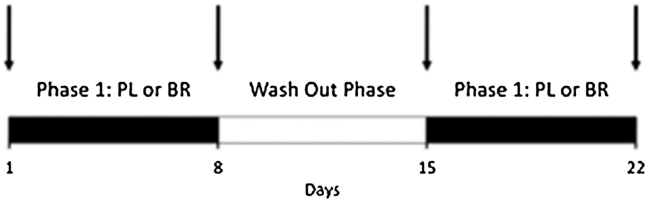

FIGURE 1—Schematic overview of the experimental protocol. Subjects completed two 8-d supplementation phases separated by a 4 ± 4 wk washout period. On the first and eighth days of supplementation for each phase, subjects completed a submaximal treadmill run and individual 1500-m running TT (denoted by ↓).

taste, smell, or appearance. The supplementation order was counterbalanced such that four subjects began supplementing with BR and four began with PL. To test the acute and chronic effects of BR supplementation, subjects completed a submaximal treadmill run and 1500-m TT on days 1 and 8 of each phase. On days 1 and 8, subjects consumed 210 mL of BR (19.5-mmol NO_3^-) or PL 2.5 h before the 1500-m TT. Subjects were instructed to consume the drink within 20 min. On days 2–7, subjects consumed 140 mL of BR (13.0-mmol NO_3^-) or PL with lunch. Experimental trials were separated by at least 7 d to ensure that the subjects had adequate recovery and to minimize disturbances in the subjects' training routines.

Dietary and training standardization. Subjects were instructed to refrain from using antibacterial mouthwash and chewing gum during the supplementation period because these have been shown to disrupt nitrite bioavailability by killing the bacteria in the mouth required to convert nitrate to nitrite (12,31). In addition, subjects were asked to abstain from using BR or other supplements during the study. However, subjects were not instructed to reduce their intake of nitrate-rich foods so that the study reflects the most practical application of BR supplementation. This is consistent with previous work (20). Before the exercise tests, subjects were advised to eat and drink as they normally would when preparing for a competition. Subjects recorded their food intake for the 36 h preceding the practice trial and were asked to replicate this diet for all subsequent experimental trials. Subjects ingested a diet abundant in CHO so that glycogen supply would not be limited during exercise. Urine specific gravity was measured upon arrival at the laboratory and confirmed that subjects arrived similarly hydrated for all trials (1.014 ± 0.006). Training during the 3 d before the first exercise test was recorded and replicated as closely as possible with respect to intensity and volume for subsequent trials. In the 24 h before the exercise test, subjects were asked not to complete any exhaustive exercise and train as though preparing for a competition. Exercise tests were performed on the same day of the week and at the same time of the day to maintain the subject's normal training routine.

Exercise tests. Subjects arrived at the laboratory (20.9°C ± 0.4°C, 22% ± 2% relative humidity) 1.5 h after ingesting the BR or PL drink to begin the submaximal treadmill run (Fig. 2). The run was designed to simulate a typical precompetition warm-up and allow for $\dot{V}O_2$ measurements at three different running speeds. The run consisted of 19 min of continuous treadmill running. Subjects ran for 7 min at 50% $\dot{V}O_{2peak}$, followed without stopping by 7 min of running at 65% $\dot{V}O_{2peak}$ and 5 min at 80% $\dot{V}O_{2peak}$. The running speed corresponding to 50%, 65%, and 80% $\dot{V}O_{2peak}$ was the same in all four trials. Running speed measurements were 10.7 ± 1.3, 14.2 ± 1.2, and 17.5 ± 1.3 $km \cdot h^{-1}$ for 50%, 65%, and 80% $\dot{V}O_{2peak}$, respectively. The treadmill incline was set at 1% to simulate running on a level surface due to lack of air drag while running on a treadmill (18). Ventilation and expired oxygen and carbon dioxide concentration were measured for the duration of the treadmill run, and measurements were averaged over 40-s intervals. Errant breaths caused by coughing or swallowing were adjusted after visual inspection. At each running intensity, $\dot{V}O_2$, $\dot{V}CO_2$, and RER were averaged over the last 120 s at that running speed. HR was recorded, with 60 s remaining at each running speed (RS4000sd; Polar, Kempele, Finland). Immediately after the treadmill run, subjects walked from the laboratory to the indoor track (17.7°C ± 1.9°C, 27% ± 8% relative humidity) and were given time to complete their warm-up routine consisting of dynamic stretching and strides. At 2.5 h after BR or PL ingestion, subjects began the individual 1500-m TT run on an indoor 200-m track (Fig. 2). Subjects were told

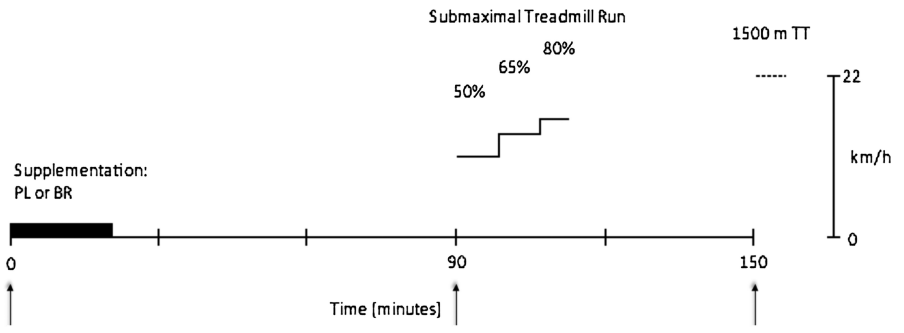

FIGURE 2—Schematic overview of the exercise test. On four separate occasions, subjects completed a submaximal treadmill run at three increasing speeds corresponding to 50%, 65%, and 80% of maximal oxygen uptake, followed by an individual 1500-m TT on an indoor track. Blood sampling is denoted by ↑.

the number of laps remaining in the run. To minimize variability in pacing strategy, subjects were given their elapsed time at 200, 400, and 600 m only (28). No further feedback regarding performance was given. Subjects were not allowed to wear their own watches during the TT. Subjects received standard encouragement to complete the TT as quickly as possible, and the time to complete the 1500-m distance was recorded. After the TT, subjects completed a questionnaire to determine whether they were blinded to the supplementation condition.

Blood sampling. Blood sampling was carried out at a separate time after the exercise testing so as not to affect the TT performances. The same eight subjects again prepared for the trial (including dietary control) and supplemented for 8 d as described previously but only with BR. On days 1 and 8, subjects arrived at the laboratory and an approximately 4-mL baseline blood sample (t = 0) was collected from the antecubital vein into a sodium-heparinized tube. On day 8, subjects arrived 23.5 ± 1.5 h after supplementation with BR the day before. After the initial blood sampling on both days, subjects were provided with 210 mL of BR to be ingested within 20 min. At 1.5 h, a second blood sample was collected just before the subjects completed the submaximal treadmill run. A final blood sample was collected at 2.5 h after ingestion, corresponding to the time at which the 1500-m run would be completed. However, for the blood sampling procedure, subjects did not complete the 1500-m TT. The $\dot{V}O_2$ data from days 1 and 8 of testing during these "blood collection trials" are not reported in the article. All blood samples were centrifuged for 4 min at 10,000 rpm. The plasma was collected and filtered using a centrifuge filter tube (Amicon Bioseparations; Millipore, Billerica, MA) with a molecular weight cutoff of 30 kD and spun for 10 min at 14,000*g*. The filtered plasma was collected and frozen at −80°C for later analysis.

Plasma analysis. Plasma samples were analyzed for nitrate + nitrite (NO_X) concentrations. Nitrate concentrations in the blood are in the micromolar range, whereas nitrite concentrations are in the nanomolar range. Therefore, plasma NO_X very closely represents plasma nitrate levels. Filtered plasma samples were analyzed fluorometrically for NO_X content using a commercially available nitrate/nitrite assay kit (item number 780051; Caymen Chemical, Ann Arbor, MI). Briefly, plasma samples were appropriately diluted with assay buffer (20-mM KH_2PO_4; pH, 7.4) and incubated for 2.5 h with nitrate reductase and the enzyme cofactor to convert all plasma nitrate to nitrite. After incubation, 2,3-diaminonaphthalene was added for the fluorometric detection of nitrite. A spectrofluorometer (SpectraMax M2e; Molecular Devices, Sunnyvale, CA) was used to determine nitrite concentration at an excitation wavelength of 365 nm and emission wavelength of 430 nm. Attempts to measure plasma [NO_2^-] were also made, but reliable results could not be obtained with this technique and were not reported.

Statistical analysis. Differences in plasma NO_X, $\dot{V}O_2$, $\dot{V}CO_2$, RER, and HR were analyzed using two-way (condition × time) ANOVA. Differences in the time to complete the 1500-m TT were assessed using one-way ANOVA. Statistical tests were performed using StatPlus (AnalystSoft, Irvine, CA). Statistical significance was accepted when $P < 0.05$. All data are presented as mean ± SD.

RESULTS

Plasma [NO_3^-]. Baseline plasma [NO_3^-] on day 1 (acute) before any supplementation was 37 ± 15 μM (Fig. 3). After BR ingestion, plasma [NO_3^-] increased significantly from baseline to 615 ± 151 μM at 90 min and 569 ± 64 μM at 150 min. There was no significant difference in plasma [NO_3^-] between 90 and 150 min. After chronic BR supplementation (day 8), baseline plasma [NO_3^-] was 270 ± 182 μM (Fig. 3). Plasma [NO_3^-] was significantly increased from baseline to 870 ± 259 μM at 90 min and 842 ± 243 μM at 150 min after ingestion. There was no significant difference in plasma [NO_3^-] between 90 and 150 min. In addition, plasma [NO_3^-] was significantly greater in the chronic compared with that in the acute condition at all time points.

1500-m TT performance. The time to complete the 1500-m individual TT was 250.7 ± 4.3 s after acute BR supplementation, 250.5 ± 6.2 s after chronic BR supplementation, 250.4 ± 7.0 s after acute PL supplementation, and 251.4 ± 7.6 s after chronic PL supplementation (Fig. 4). There was no significant difference in the time to complete the 1500-m run between any of the four conditions. In addition, no significant order effect was observed in the time to complete the 1500-m run regardless of the condition (249.4 ± 6.2, 249.7 ± 6.0, 251.8 ± 4.8, and 252.0 ± 7.7 s for trials 1, 2, 3, and 4, respectively). Interestingly, two potential "responders" to BR supplementation were identified in the group of elite runners. These two subjects had improved 1500-m run performance after the acute and chronic BR supplementation compared with that after acute and chronic

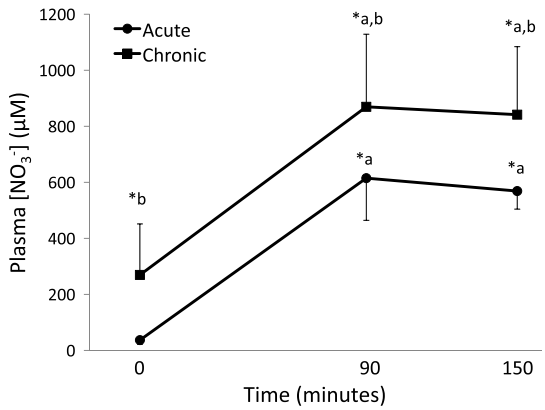

FIGURE 3—Plasma [NO_3^-] at baseline (t = 0 min) and 90 and 150 min after ingestion of 210 mL (19.5-mmol NO_3^-) of concentrated BR for acute and chronic supplementation. For both acute and chronic supplementation, plasma [NO_3^-] was significantly greater at 90 and 150 min compared with that at baseline (*a). At all time points, plasma [NO_3^-] was significantly greater for chronic compared with that for acute supplementation (*b). Values are mean ± SD.

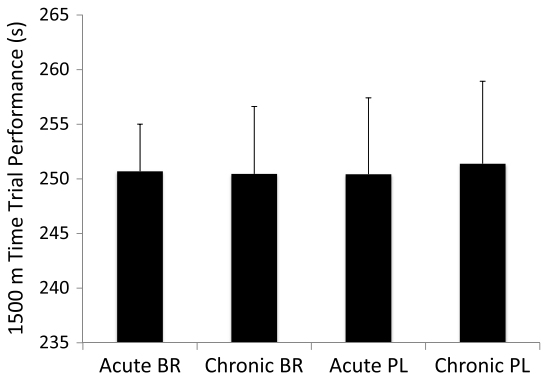

FIGURE 4—The effects of acute and chronic BR and PL supplementation on 1500-m running TT performance. No significant difference was observed between any condition. Values are mean ± SD.

PL supplementation. Performance was improved by 5.8 and 5.0 s (acute) and 7.0 and 0.5 s (chronic) for the two responders.

Submaximal treadmill run. There were no significant differences in $\dot{V}O_2$ at any time between any of the four supplementation conditions during treadmill running at speeds corresponding to 50%, 65%, and 80% of $\dot{V}O_{2peak}$ (Fig. 5). In addition, $\dot{V}CO_2$, RER, and HR were unchanged at all three running speeds between all four supplementation conditions (Table 1). Regardless of condition, $\dot{V}O_2$, $\dot{V}CO_2$, and HR were increased during running at 65% $\dot{V}O_{2peak}$ compared with that at 50% and further increased for running at 80% compared with that at 50% and 65%. RER was significantly greater during running at 80% $\dot{V}O_{2peak}$ compared with that at 50% and 65%, with no difference between the 50% and 65% intensities. It should be noted that $\dot{V}O_2$ also tended to be decreased for the two BR supplementation responders mentioned previously. For the first, $\dot{V}O_2$ was unchanged at 50% $\dot{V}O_{2peak}$ but decreased by 52 mL (acute) and 88 mL (chronic) at 65% $\dot{V}O_{2peak}$ and decreased by 211 mL (acute) or did not change (chronic) for running at 80% $\dot{V}O_{2peak}$ after BR supplementation. The second responder had 42- and 157-mL decreases in $\dot{V}O_2$ at 50% $\dot{V}O_{2peak}$, 96- and 210-mL reductions at 65%, and 25- and 66-mL decreases in $\dot{V}O_2$ for running at 80% $\dot{V}O_{2peak}$ after acute and chronic BR supplementation, respectively.

Supplementation blinding. Subjects could not consistently distinguish between the nitrate-depleted PL drink and the nitrate-rich BR drink. When asked which beverage they had consumed after each trial, subjects responded that they were unsure 14 of 32 times. When athletes believed that they knew what they consumed, they correctly identified the drink nine times but responded incorrectly nine times.

DISCUSSION

The principal finding of this study was that nitrate supplementation with BR rich in NO_3^- did not improve submaximal treadmill running economy or 1500-m TT performance of elite distance runners. This is the first study to test the effects of BR supplementation in elite distance runners ($\dot{V}O_{2peak}$, >75 mL·kg^{-1}·min^{-1}) using a running-specific performance test. The lack of effect of BR supplementation on performance is consistent with recent publications in well-trained competitive cyclists and cross-country skiers (5,9,10,28,33).

In recreationally active and moderately trained subjects, nitrate supplementation improved exercise economy and performance, potentially by reducing the adenosine triphosphate cost of contraction and/or improving mitochondrial respiratory efficiency via increased NO bioavailability (2,26). However, the findings of the current study support the idea that in well-trained individuals, the training adaptations that accompany endurance exercise diminished the possible benefit of nitrate supplementation (5,10,11,30,32). It is possible that nitrate supplementation fails to elicit a

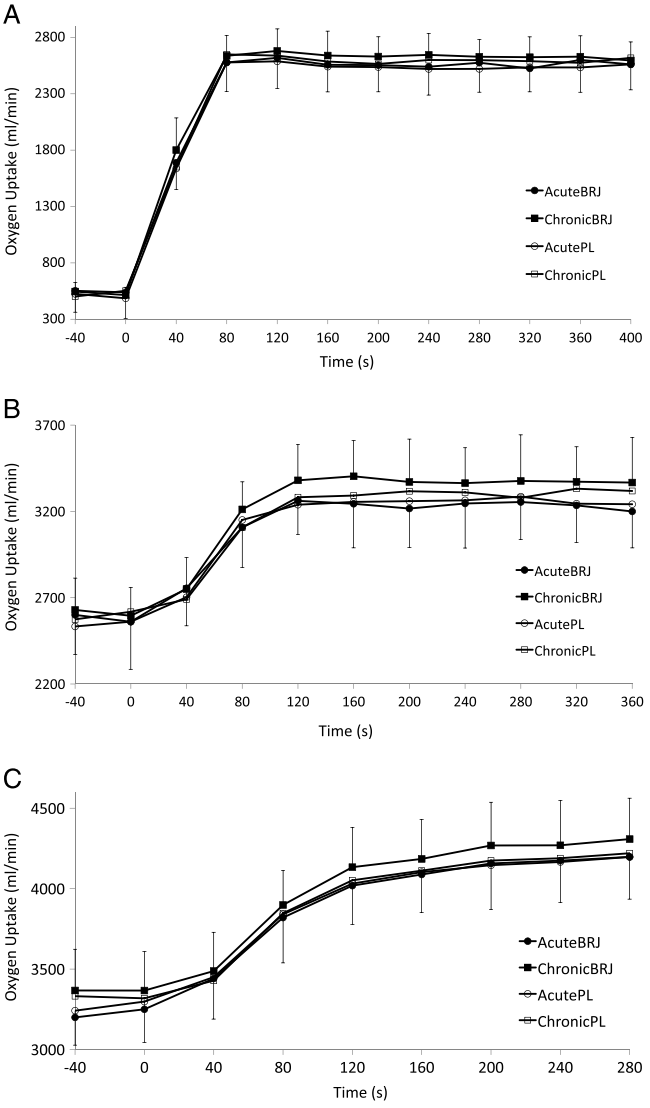

FIGURE 5—Oxygen uptake for subjects running at 50% $\dot{V}O_{2peak}$ (10.6 ± 1.3 km·h^{-1}) (A), 65% $\dot{V}O_{2peak}$ (14.0 ± 1.2 km·h^{-1}) (B), and 80% $\dot{V}O_{2peak}$ (17.5 ± 1.3 km·h^{-1}) (C) after acute and chronic BR and PL supplementation. No significant difference between any condition was observed at any time. Values are mean ± SD.

TABLE 1. Physiological responses to running at 50%, 65%, and 80% of $\dot{V}O_{2peak}$ after acute and chronic PL and BR supplementation.

	Intensity (% $\dot{V}O_{2peak}$)		
	50%	65%	80%
$\dot{V}O_2$ (mL·min^{-1})			
Acute PL	2537 ± 213	3265 ± 242	4198 ± 257
Chronic PL	2596 ± 206	3315 ± 240	4224 ± 273
Acute BR	2561 ± 216	3239 ± 214	4195 ± 322
Chronic BR	2630 ± 173	3367 ± 225	4309 ± 268
$\dot{V}CO_2$ (mL·min^{-1})			
Acute PL	2160 ± 257	2904 ± 301	3851 ± 367
Chronic PL	2257 ± 199	3001 ± 255	3992 ± 383
Acute BR	2173 ± 250	2881 ± 272	3876 ± 433
Chronic BR	2233 ± 228	2944 ± 307	3948 ± 411
RER			
Acute PL	0.85 ± 0.06	0.89 ± 0.05	0.92 ± 0.05
Chronic PL	0.87 ± 0.02	0.89 ± 0.06	0.94 ± 0.04
Acute BR	0.85 ± 0.04	0.89 ± 0.04	0.92 ± 0.04
Chronic BR	0.85 ± 0.06	0.87 ± 0.06	0.92 ± 0.05
HR (bpm)			
Acute PL	130 ± 18	149 ± 21	173 ± 17
Chronic PL	132 ± 15	156 ± 19	178 ± 16
Acute BR	129 ± 14	151 ± 15	173 ± 13
Chronic BR	133 ± 17	155 ± 18	176 ± 12

Values are mean ± SD.
Measurements for $\dot{V}O_2$, $\dot{V}CO_2$, and RER are the average of the last 120 s at each running intensity. HR was taken with 60 s remaining at each intensity. There were no significant differences between any conditions.

response in well-trained subjects because supplementation does not augment the use of nitrite from endogenous sources for the production of NO during exercise. In other words, there is a threshold where further increases in NO_2^- via dietary nitrate supplementation will not confer additional improvements.

Interestingly, two recent reports have examined the effects of BR supplementation in well-trained rowers. In a study by Bond et al. (6), junior male rowers with at least one season of rowing experience improved their performance during maximal repeated 6 × 500 m rowing ergometer bouts. However, it is unclear whether these results would be found in a competitive setting (e.g., 2000-m distance) and with well-trained adult rowers. In a recent study with highly trained adult male rowers, the authors concluded that BR supplementation had no effect on 2000-m rowing TT performance for a moderate BR dose (4.2-mmol NO_3^-) and may improve performance for a high dose (8.4-mmol NO_3^-) (15).

Nitrite utilization. As discussed previously (10,32), dietary sources of nitrite may not enhance NO bioavailability in well-trained individuals for two reasons. First, trained individuals exhibit higher baseline plasma [NO_3^-] compared with untrained individuals. Plasma nitrate was 41% higher in endurance athletes compared with that in untrained control subjects (23.2 vs 16.4 μM, respectively) (29), and exercise training has been shown to increase endothelial NOS expression and activity, leading to higher plasma [NO_2^-] and [NO_3^-] (13). Second, endurance training may diminish the reliance on the nitrate–nitrite–NO pathway during exercise because the development of hypoxic loci within the skeletal muscle may be reduced (32). Potential improvements in muscle perfusion and O_2 distribution afforded by nitrate supplementation may not be observed in well-trained subjects because endurance training increases skeletal muscle capillary density (7). Therefore, blood flow and O_2 distribution may already be optimal in well-trained individuals. Unfortunately, determining an individual's capacity to utilize nitrite is difficult, considering that plasma [NO_2^-] is a product of nitrate reduction and NO oxidation from the classical NOS pathway (33). Regardless, high basal [nitrate] and [nitrite] and/or diminished reliance on nitrite during exercise in well-trained individuals may reduce the efficacy of nitrate supplementation.

Plasma [nitrate]. Previous studies on well-trained subjects have suggested that these individuals may require greater nitrate exposure via a higher dose and/or increased supplementation duration to see a performance benefit (5,9,10,28,32). In the current study, baseline plasma [NO_3^-] before acute supplementation was 37 ± 15 μM and similar to the average of those in five studies on well-trained subjects (37 ± 6 μM) (4,5,9,10,28). With supplementation of 5- to 11-mmol NO_3^- in previous studies on well-trained subjects, plasma [NO_3^-] increased to 261 ± 66 μM at 2.5 h after ingestion (Δ[NO_3^-] from baseline, 224 μM). In the present study, subjects were provided a dose of 19.5-mmol NO_3^-, which is nearly twice the highest dose reported in studies on well-trained subjects. Accordingly, the increase in plasma [NO_3^-] was more substantial, as plasma [NO_3^-] increased to 569 ± 64 and 842 ± 243 μM at 2.5 h after ingestion for acute and chronic supplementation, respectively (Δ[NO_3^-] from baseline, 532 and 572 μM for acute and chronic supplementation, respectively). Although we acknowledge that we did not measure [NO_2^-], no improvement in exercise economy or performance was observed in the present study despite the larger increase in plasma [NO_3^-]. In addition, we directly compared the effects of acute and chronic (8 d) nitrate supplementation. Interestingly, plasma [NO_3^-] had not returned to presupplementation levels 24 h after BR ingestion, the day before subjects arrived at the laboratory for baseline blood sampling during chronic supplementation (37 ± 15 and 270 ± 182 μM, respectively). This finding indicates that plasma [NO_3^-] exposure was significantly increased for the duration of the chronic supplementation. However, this increased nitrate exposure did not reduce the O_2 cost of submaximal treadmill running or improve the time to complete a 1500-m running TT. This evidence supports the idea that increased plasma [NO_3^-] and most likely [NO_2^-] via dietary supplementation may not improve economy or performance because nitrite availability may already be high from endogenous sources in well-trained individuals.

TT duration. Evidence in recreationally active subjects predicts that the ergogenic effects of BR supplementation are maximized for TT lasting approximately 4 min (19). Despite this fact, our results show that the time to complete a 1500-m run, which took the subjects 4 min 10 s, was not improved after BR supplementation. In addition, other studies on well-trained subjects have failed to show improved performance during TT lasting approximately 15 (28), 20 (10), 40 (5), and 60 (9) min and 2 h 15 min (32)

after nitrate supplementation. Taken together, these observations suggest that the efficacy of nitrate supplementation in well-trained subjects is not dependent on TT length because nitrate ingestion failed to improve performance over a wide range of TT durations.

Individual responders. Although mean $\dot{V}O_2$ and TT performance were unaffected by BR supplementation, two potential "responders" were identified in the group of elite runners. These were the only two subjects to improve their 1500-m run performance after both the acute (5.8 and 5.0 s) and chronic (7.0 and 0.5 s) BR supplementation compared with that after acute and chronic PL supplementation. The coefficient of variation (CV) for 1500-m running performance of elite males is 0.9% (16), and the smallest worthwhile enhancement in performance of elite 1500-m runners is 0.3–0.5 of the CV (17). This corresponds to a 0.68- to 1.13-s improvement in the time to complete the 1500-m run in the current study. Therefore, the performance improvement after BR supplementation could be practically meaningful during actual competition for the two responders.

$\dot{V}O_2$ also tended to be decreased for the two responders. For one responder, $\dot{V}O_2$ was unchanged at 50% $\dot{V}O_{2peak}$ but decreased by 52 mL (acute) and 88 mL (chronic) at 65% $\dot{V}O_{2peak}$ and decreased by 211 mL (acute) or did not change (chronic) for 80% $\dot{V}O_{2peak}$ after BR supplementation. The other responder had 42- and 157-mL $\dot{V}O_2$ decreases at 50% $\dot{V}O_{2peak}$, 96- and 210-mL reductions at 65% $\dot{V}O_{2peak}$, and 25- and 66-mL decreases at 80% $\dot{V}O_{2peak}$ after acute and chronic BR supplementation, respectively. In the current study, the CV for $\dot{V}O_2$ between the acute and chronic PL trials, where presumably there was no effect of condition, were 3%, 4%, and 5% for running at 50%, 65%, and 80% of $\dot{V}O_{2peak}$, respectively. The corresponding values of measurement variability for $\dot{V}O_2$ were 70, 163, and 164 mL at 50%, 65%, and 80%, respectively. For the two responders, the magnitude of the $\dot{V}O_2$ reduction was greater than the measurement variability at some times but not always. Therefore, repeated testing of these two subjects could determine whether a true reduction in $\dot{V}O_2$ after BR supplementation is present.

These results support the findings of Christensen et al. (10) who also identified two "responders" out of a group of eight highly trained cyclists. Similarly, the only two subjects to demonstrate improved exercise economy during submaximal cycling at 50% and 70% of the incremental test peak power were also the only subjects who had improved TT performances. Taken together, these findings are important for two reasons. First, although the likelihood of seeing a response may be reduced, it is possible that elite runners and cyclists can benefit from nitrate supplementation. Second, improved performance was seen in individuals who had increased submaximal exercise economy. In the current study, responders could not be distinguished by baseline plasma $[NO_3^-]$ or increases after supplementation. However, the two responders tended to have slower 1500-m personal best measurements (fifth and sixth fastest out of eight) and lower $\dot{V}O_{2peak}$ (sixth and eighth highest out of eight, 80.1 and 69.2 $mL \cdot kg^{-1} \cdot min^{-1}$, respectively) compared with those of other subjects in the study. In addition, the two responders had lower self-reported training volume for both hours per week and number of years (sixth and eighth highest out of eight). It is possible that BR ingestion improved performance of the two responders in the present study because they were less adapted to endurance training compared with the other subjects. Further research is required to fully explain the observed individual variability after nitrate supplementation.

Limitations. One limitation of the current study is that plasma $[NO_X]$, which closely approximates $[NO_3^-]$, was measured rather than $[NO_2^-]$. It is possible that subjects who had increased plasma $[NO_3^-]$ did not have a subsequent increase in plasma $[NO_2^-]$. However, the possibility of this occurrence seems unlikely, given the large dose of nitrate ingested. In any case, this may have inhibited our ability to determine the reason why some subjects may not respond to nitrate supplementation. No change in plasma $[NO_2^-]$ may preclude individuals from responding positively to BR ingestion (32). However, this did not limit the ability to detect responders because baseline plasma $[NO_2^-]$ and increases after supplementation cannot be distinguished between responders and nonresponders (5,10). Rather, an improvement in submaximal exercise economy may be the best way to predict who will have improved TT performance (10).

In the current study, the CV for $\dot{V}O_2$ between the acute and chronic PL trials, where presumably there was no effect of condition, were 3%, 4%, and 5% for running at 50%, 65%, and 80% of $\dot{V}O_{2peak}$, respectively. So, the change in $\dot{V}O_2$ after BR supplementation would have to be greater than 70, 163, and 164 mL at 50%, 65%, and 80%, respectively, to detect an effect. Therefore, the current methodology may not be sufficiently sensitive to detect small reductions in $\dot{V}O_2$ after nitrate supplementation. It should also be mentioned that the treadmill test for the $\dot{V}O_2$ measurements started 90 min after BR ingestion. This may be earlier than the time at which plasma nitrite would be elevated, and $\dot{V}O_2$ changes might be expected (33). Also, exercise causes a reduction in plasma nitrite (although nitrate remains relatively stable). To what extent the previous $\dot{V}O_2$ economy tests affected the nitrite levels before the TT is therefore not known, but we believe that the short duration of the $\dot{V}O_2$ tests and the large ingested dose of BR reduced the likelihood that these issues were a concern.

The present study used a 1500-m individual TT to assess performance, which has been shown to have higher practical validity and reliability compared with time-to-exhaustion tests (11). The testing protocol was very repeatable for the elite trained subjects of the current study, as the CV for all trials was 0.97% and matches that reported for an actual competition for elite male runners (0.9%) (16).

CONCLUSIONS

The principal finding of the present study was that supplementation with nitrate-rich BR did not improve submaximal treadmill running economy or 1500-m TT performance of eight elite male distance runners when examined on a group basis. This finding is the first to investigate the effects of nitrate supplementation in elite runners using a running-specific test of performance. The lack of improvement in exercise economy or performance observed in the current study corroborates recent observations in well-trained cyclists and cross-country skiers (5,9,10,28,32). Overall, the lack of improvement in exercise economy or performance could not be attributed to insufficient nitrate exposure because plasma [NO_3^-] after BR ingestion in the current study was 2.2- and 3.3-fold greater for acute and chronic supplementation, respectively, than those previously reported in studies on well-trained subjects. Importantly, two responders in our group of elite runners were identified. This indicates that BR supplementation may be effective in a small proportion of elite athletes, and the present results are consistent with Christensen et al. (10) in suggesting that approximately 25% of elite athletes might benefit.

The funding for this research was received from the Natural Sciences and Engineering Research Council of Canada.

There is no conflict of interest with any financial organization regarding the material discussed in the article.

The results of the present study do not constitute endorsement by the American College of Sports Medicine.

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