Acute Dietary Nitrate Supplementation Improves Cycling Time Trial Performance

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ABSTRACT

LANSLEY, K. E., P. G. WINYARD, S. J. BAILEY, A. VANHATALO, D. P. WILKERSON, J. R. BLACKWELL, M. GILCHRIST, N. BENJAMIN, and A. M. JONES. Acute Dietary Nitrate Supplementation Improves Cycling Time Trial Performance. Med. Sci. Sports Exerc., Vol. 43, No. 6, pp. 1125–1131, 2011. Purpose: Dietary nitrate supplementation has been shown to reduce the \( O_2 \) cost of submaximal exercise and to improve high-intensity exercise tolerance. However, it is presently unknown whether it may enhance performance during simulated competition. The present study investigated the effects of acute dietary nitrate supplementation on power output (PO), \( VO_2 \), and performance during 4- and 16.1-km cycling time trials (TT). Methods: After familiarization, nine club-level competitive male cyclists were assigned in a randomized, crossover design to consume 0.5 L of beetroot juice (BR; containing \( \sim 6.2 \) mmol of nitrate) or 0.5 L of nitrate-depleted BR (placebo, PL; containing \( \sim 0.0047 \) mmol of nitrate), \( \sim 2.5 \) h before the completion of a 4- and a 16.1-km TT. Results: BR supplementation elevated plasma [nitrite] (\( PL = 241 \pm 125 \) vs BR = 575 \( \pm 199 \) nM, \( P < 0.05 \)). The \( VO_2 \) values during the TT were not significantly different between the BR and PL conditions at any elapsed distance (\( P > 0.05 \)), but BR significantly increased mean PO during the 4-km (\( PL = 279 \pm 51 \) vs BR = 292 \( \pm 44 \) W, \( P < 0.05 \)) and 16.1-km TT (\( PL = 233 \pm 43 \) vs BR = 247 \( \pm 44 \) W, \( P < 0.01 \)). Consequently, BR improved 4-km performance by 2.8% (\( PL = 6.45 \pm 0.42 \) vs BR = 6.27 \( \pm 0.35 \) min, \( P < 0.05 \)) and 16.1-km performance by 2.7% (\( PL = 27.7 \pm 2.1 \) vs BR = 26.9 \( \pm 1.8 \) min, \( P < 0.01 \)). Conclusions: These results suggest that acute dietary nitrate supplementation with 0.5 L of BR improves cycling economy, as demonstrated by a higher PO for the same \( VO_2 \) and enhances both 4- and 16.1-km cycling TT performance. Key Words: NITRIC OXIDE, EFFICIENCY, BLOOD PRESSURE, EXERCISE TOLERANCE

Dietary nitrate has recently emerged as a potential modulator of resting blood pressure (22,38,40) and muscle energy metabolism (1,25) and as a possible “natural” ergogenic aid to exercise performance (1,2,24). Strikingly, dietary supplementation either with pharmacological sodium nitrate or with nitrate-rich beetroot juice (BR) has been shown to result in a significant reduction in the \( O_2 \) cost of submaximal cycling (2,26,38), knee extensor exercise (1), and treadmill walking and running (24). This enhanced exercise efficiency is likely to be mediated through the metabolic conversion of inorganic nitrate to bioactive nitrite and, subsequently, nitric oxide (4,8).

It has been reported that dietary nitrate supplementation extended time-to-exhaustion during high-intensity constant-work-rate exercise by \( \sim 15\%–25\% \) (1,2,24). This effect, which can be estimated to be equivalent to an improvement in sporting performance of approximately 1%–2% (21), seems to be linked to an attenuation of the \( VO_2 \) slow component (1,2) and an associated blunting of changes in the muscle metabolic milieu (increased ADP and \( P_i \) and reduced PCr), which have been linked with the process of muscle fatigue (1). Time-to-exhaustion protocols, however, are tests of “exercise capacity” rather than of performance per se (9,29,41). Indeed, time-to-exhaustion tests have been criticized for having poor ecological validity and limited applicability to the athletic arena (9,29). A superior test of the possible efficacy of dietary nitrate as an intervention for enhancing sports performance would involve subjects completing a given distance in the fastest possible time, i.e., a time trial (TT) (9,13,33).

The purpose of the present study was therefore to investigate the effect of dietary nitrate supplementation on cycle TT performance. Specifically, we investigated the influence of acute dietary nitrate supplementation, in the form of nitrate-rich BR, on plasma [nitrite] (a biomarker of NO concentration, which can act as a regulator of \( O_2 \) delivery to working muscle (1,25) and as a modulator of skeletal muscle \( VO_2 \) (8)).
was imposed until the subject was unable to continue. The breath-by-breath pulmonary gas exchange data were collected continuously during the incremental test and averaged during consecutive 10-s periods. The \(\dot{V}O_2\)peak was taken as the highest 30-s mean value attained before the subject's volitional termination of the test.

The subjects were then familiarized with performing cycling TT in the laboratory. All TT were performed on a Computrainer cycle ergometry system (Racermate Computrainer, Seattle, WA). It has been reported that this ergometer provides a reliable measure of PO (11). Before each test, the Computrainer was calibrated in accordance with the manufacturer’s recommendations. Each subject performed familiarization of the 4- and 16.1-km (i.e., 10 miles) TT on at least two occasions for each distance. To ensure that the subjects were fully familiarized, TT were repeated for each subject until the difference in completion times was <1%.

Supplementation tests. Each subject returned to the laboratory on four occasions to complete each of the following conditions: 1) 4-km TT after nitrate-rich BR supplementation, 2) 4-km TT after nitrate-depleted PL supplementation, 3) 16.1-km TT after BR supplementation, and 4) 16.1-km TT after PL supplementation. All four conditions were administered using a randomized, double-blind, crossover design. A 48- to 72-h washout period separated each TT.

For 24 h before the first TT, subjects recorded their food and fluid intake and physical activity and were instructed to replicate these in the 24 h before subsequent TT. The subjects were instructed to arrive at the laboratory in a rested and fully hydrated state, at least 1 h postprandial, and to avoid strenuous activity in the 24 h preceding each testing session. Subjects were also asked to refrain from caffeine and alcohol 6 and 24 h before each test, respectively. The subjects also abstained from using antibacterial mouthwash and chewing gum during the supplementation periods because these are known to eradicate the oral bacteria that are necessary for the conversion of nitrate to nitrite (16).

On test days, subjects arrived at the laboratory at ~9:00 a.m. After a 10-min period of quiet rest, the blood pressure of the brachial artery was measured with subjects in a rested, seated position using an automated sphygmomanometer (Dinamap Pro; GE Medical Systems, Tampa, FL). Four measurements were recorded, and the mean of the final three measurements was used for data analysis. A venous blood sample (~4 mL) was drawn into lithium-heparin tubes (7.5-mL Monovette Lithium Heparin; Sarstedt Ltd., Leicester, United Kingdom, U.K.), which have very low levels of nitrate (0.89 ± 0.35 μM) and nitrite (0.05 ± 0.01 μM). Samples were subsequently analyzed for plasma [nitrite] (NO\(_2\)\(^-\)) using a modification of the chemiluminescence technique (3), as described previously (2).

During a 15-min period, subjects then ingested 0.5 L of either BR (organic BR containing ~6.2 mmol of NO\(_3\)\(^-\); Beet It; James White Drinks Ltd., Ipswich, United Kingdom, U.K.) or PL (organic nitrate-depleted BR containing ~0.0047 mmol of NO\(_3\)\(^-\); Beet It; James White Drinks Ltd.). The nitrate-depleted BR (as placebo), which was created by passing the juice through an ion exchange resin, which is selective for nitrate ions (24), was otherwise similar to the experimental beverage in appearance, odor, taste, and texture. This advance enabled us to isolate the effects of dietary nitrate from the other potential “active” ingredients found in BR in a genuinely double-blind experimental design (see Lansley et al. (24) for discussion).

After ingestion of the BR or PL beverage, subjects were given a period of 2 h during which they were allowed to leave the laboratory but were asked to refrain from strenuous physical activity. Subjects were also asked to fast during this time, although water was permitted ad libitum. The subjects returned to the laboratory at ~11.30 a.m., and blood pressure measurements and venous blood samples were repeated. The subjects then completed either a 4- or 16.1-km TT approximately 2.75 h after BR or PL ingestion. This period...
was chosen as peak plasma \([\text{NO}_2^-]\) concentrations occur 2.5–3.0 h after ingestion of BR (40).

During each TT, a computer screen was placed in front of subjects, which displayed the Computrainer software program. Subjects could see a computer-projected simulation of themselves, the distance they had cycled, and the distance remaining to complete the TT. Each TT began with a stationary start after a numerical countdown. Subjects were given no indication of completion time and received no feedback on performance during or after the TT. The computrainer ergometry system recorded PO every 10 s, and these values were averaged for every 0.5 and 2.0 km completed for the 4- and 16.1-km TT, respectively, to create a PO profile. During all TT, pulmonary gas exchange and ventilation were measured continuously with subjects wearing a nose clip and breathing through a mouthpiece and impeller turbine assembly (Jaeger Triette V, Hoechburg, Germany). The inspired and expired gas volume and gas concentration signals were continuously sampled at 100 Hz, the latter using paramagnetic (\(O_2\)) and infrared (\(CO_2\)) analyzers (Jaeger Oxycon Pro) via a capillary line connected to the mouthpiece. These analyzers were calibrated before each test with gases of known concentration, and the turbine volume transducer was calibrated using a 3-L syringe (Hans Rudolph, Kansas City, MO). The \(V_O_2\) and PO data were time-aligned to the start of exercise and averaged during consecutive 10-s periods and for every 0.5 and 2.0 km completed for the 4- and 16.1-km TT, respectively. These data were also used to produce a PO/\(V_O_2\) ratio, measured in watts of PO produced per liter of \(O_2\) consumed per min (W/L\(^-1\)-min\(^{-1}\)) for each condition. The \(V_O_2^{\text{peak}}\) was taken as the highest 30-s mean value attained during the TT.

**Statistical analysis.** Differences in plasma \([\text{NO}_2^-]\), blood pressure, mean PO, mean \(V_O_2\), and completion time were assessed using Student’s paired \(t\)-tests and one-way ANOVA where appropriate. The PO, \(V_O_2\), and PO/\(V_O_2\) ratio profiles were graphed for all 4- and 16.1-km distances and analyzed using a two-way (supplement \(\times\) time) ANOVA with Bonferroni correction. A priori power analysis showed that a sample size of nine would allow detection of a 2%–3% difference in cycling TT performance with high statistical power (1 – \(\beta\) = 0.80; 0.05 = \(\alpha\) level). Subsequent effect size calculations indicate a 0.4–0.5 (medium–large) effect magnitude, calculated using Cohen \(d\). Statistical tests were conducted using SPSS version 16.0 (Chicago, IL), and significance was accepted when \(P<0.05\). A statistical spreadsheet was used to derive a mechanistic inference from the results using the \(P\) value (20). Data are reported as mean ± SD, unless stated otherwise.

**RESULTS**

**Plasma \([\text{NO}_2^-]\) and blood pressure.** The group mean plasma \([\text{NO}_2^-]\) values before supplementation were 293 ± 133 nM, which is within the reference range for healthy humans. No significant changes were observed in plasma \([\text{NO}_2^-]\) after PL supplementation (PL = 241 ± 125 nM, \(P > 0.05\)). However, relative to placebo, BR ingestion increased plasma \([\text{NO}_2^-]\) by 138% (BR = 575 ± 199 nM, \(P < 0.05\)). Compared with presupplementation values, PL supplementation had no significant effect on systolic, diastolic, or mean arterial blood pressure. However, the ingestion of BR significantly reduced systolic blood pressure relative to placebo (PL = 131 ± 8 vs BR = 125 ± 5 mm Hg, \(P<0.01\)). Diastolic blood pressure and mean arterial pressure, which were 72 ± 4 and 93 ± 4 mm Hg at presupplementation baseline, respectively, were not significantly altered by BR ingestion.

**4-km TT performance.** The mean 4-km completion time obtained during the familiarization trials was 6.43 ± 0.42 min. No significant changes were observed after PL supplementation (PL = 6.45 ± 0.42 min, \(P > 0.05\)). However, relative to PL, BR ingestion reduced completion time in all nine subjects, with a group mean reduction of 2.8% (BR = 6.27 ± 0.35 min, \(P < 0.05\)). The mean and individual 4-km completion times are displayed in Figure 1A, and the accompanying PO profiles are shown in Figure 1B. The ingestion of BR increased mean PO by 5% (PL = 279 ± 51 vs BR = 292 ± 44 W, \(P < 0.05\)). Statistical inferences (20) indicated a 98.8% probability that the true value of the effect statistic would be practically meaningful to an athlete.

**Analyses of the \(V_O_2\) response revealed no significant differences between PL and BR across elapsed distance (\(P > 0.05\)). Consequently, BR supplementation increased the mean PO/\(V_O_2\) ratio by 11% (PL = 83 ± 9 vs BR = 93 ± 17 W/L\(^{-1}\)-min\(^{-1}\), \(P < 0.05\)).** The PO/\(V_O_2\) ratio profiles after PL and BR supplementation are shown in Figure 1C; ANOVA revealed a significant main effect for supplement (\(P < 0.05\)) and differences between PL and BR at five of six elapsed distances (\(P < 0.01\)). There was no difference in \(V_O_2\) between conditions (PL = 4.36 ± 0.47 vs BR = 4.46 ± 0.50 L/min\(^{-1}\), \(P > 0.05\)), and the values did not differ significantly from the \(V_O_2^{\text{peak}}\) measured during the ramp incremental test (\(P > 0.05\)).

**16.1-km TT performance.** The mean 16.1-km completion time obtained during the familiarization trials was 28.6 ± 2.4 min, and no significant changes were observed after PL supplementation (PL = 27.7 ± 2.1 min, \(P > 0.05\)). However, relative to PL, BR supplementation reduced 16.1-km completion time in all nine subjects, with a group mean reduction of 2.7% (BR = 26.9 ± 1.8 min, \(P < 0.01\)). The mean and individual 16.1-km completion times are displayed in Figure 2A, and the accompanying PO profiles are shown in Figure 2B. BR supplementation increased mean PO by 6% (PL = 233 ± 43 vs BR = 247 ± 44 W, \(P < 0.01\)). ANOVA revealed a significant main effect for supplement (\(P < 0.01\)) and a higher PO after BR at four of eight elapsed distances (\(P < 0.01\)). Mechanistic inferences indicated a 99.9% probability that the observed effect would be practically meaningful to an athlete.

**Analyses of the \(V_O_2\) response revealed no significant differences between PL and BR across elapsed distance (\(P > 0.05\)).**
Therefore, BR supplementation increased the mean PO/V\(\dot{O}_2\) ratio by 7% (PL = 64 ± 6 vs BR = 69 ± 3 W/L/min, \(P < 0.05\); Fig. 2C). There was no difference in VO\(_{2}\)peak between conditions (PL = 4.19 ± 0.56 vs BR = 4.23 ± 0.47 L/min, \(P > 0.05\)), but both values were significantly lower than the VO\(_{2}\)peak measured during the ramp incremental test (\(P < 0.05\)).

**DISCUSSION**

The principal finding of this investigation was that acute dietary nitrate supplementation improved 4- and 16.1-km TT performance in competitive cyclists by 2.8% and 2.7%, respectively. This effect was appreciably greater than the variability in TT performance (<1%) as established during familiarization sessions. The improved TT performance after ingestion of nitrate-rich BR was consequent to a significantly greater PO for the same VO\(_2\). In contrast, dietary supplementation with nitrate-depleted BR did not alter plasma [nitrite], PO, or TT completion time, relative to the non-supplemented control values. These data indicate that the physiological effects of BR supplementation on exercise economy and performance are consequent to its high nitrate content.

**Effects of dietary nitrate on plasma [nitrite] and blood pressure.** BR supplementation increased plasma...
[\text{NO}_3^-] \) (a biomarker of nitric oxide availability) (27,28,35) by 138%–2.5 h after ingestion and resulted in a 6-mm Hg (5%) reduction in systolic blood pressure in the young, healthy subjects who participated in this study. This is consistent with previous acute dietary nitrate interventions (22,38,40). In contrast, neither plasma [\text{NO}_3^-] nor systolic blood pressure was significantly altered after PL supplementation. This suggests that the physiological effects observed after BR were mediated through the systemic reduction of nitrate-derived nitrite to the potent vasodilator and signaling molecule, nitric oxide (4,8,12,31).

**Effects of dietary nitrate on cycling TT performance.** This is the first study to investigate the effects of dietary nitrate supplementation on athletic performance. Earlier reports indicated that nitrate supplementation reduced the O$_2$ cost of submaximal exercise and enhanced exercise tolerance, as assessed by time-to-exhaustion during high-intensity constant-work-rate exercise (1,2,24) or peak PO attained during incremental exercise after chronic supplementation (38). The use of a time-to-exhaustion protocol was valuable in these studies to establish the effect of the intervention upon the iso-time VO$_2$ response to constant-work-rate exercise. However, its use has significant limitations. Time-to-exhaustion tests are primarily measures of “exercise capacity,” and because there is no competitive sports event in which the goal is to keep going for as long as possible, tests of this type have limited ecological validity (9). In addition, it has been reported that there is no relationship between measures of time-to-exhaustion and actual cycling performance (29). In contrast, the TT protocol used in the present study has a high ecological validity (9), provides an accurate simulation of the physiological responses during competition (13), and correlates well with actual race performance (33). A further strength of the present study was the use of a nitrate-depleted beverage as a placebo; this ensured that the study design was double-blind and reduced the possibility that the results were influenced by a potential “placebo” effect.

The most striking finding of the present investigation was the significant improvement in 4- and 16.1-km TT performance after the ingestion of a single 0.5-L BR beverage, with all nine individuals completing both distances faster after BR supplementation. These findings indicate that, at least under the conditions of the present study, dietary nitrate supplementation has the potential to benefit athletic performance, at least in events of ~5–30 min in duration. To more effectively determine whether BR supplementation would be practically valuable in athletic competition, we used another statistical approach to derive the “true effect” of the intervention (20). Results showed that the improvements in TT performance after dietary nitrate supplementation were 98.9%–99.9% likely to be “beneficial or substantially positive” to an athlete. Consequently, the present findings suggest that acute BR supplementation might have both a statistically significant and practically meaningful benefit to athletic performance. Indeed, the 2.7%–2.8% improvement in TT performance observed after BR supplementation is considerably greater than the “smallest worthwhile change” (~0.6%) for road TT cyclists proposed by Paton and Hopkins (34). However, it remains to be determined whether dietary nitrate supplementation can enhance performance by this magnitude in elite cyclists.

It is important to note that we have used BR as a supplement in the present study and in our previous work (1,2,24,38) because it is a practical expedient for acutely increasing dietary nitrate intake. Although the magnitude of the improvement in performance after consumption of a natural vegetable juice beverage might seem surprising, it is important to note that the acute dose of nitrate used in the present study (i.e., 0.5 L of BR containing ~6.2 mmol of nitrate) is 4–12 times greater than the typical daily dietary nitrate intake in the United States (32). The nitrate content of foodstuffs can vary considerably (17). However, the consumption of 0.5 L of BR enables a substantial nitrate load to be ingested quickly and easily before training or competition.

In the present investigation, we used a placebo beverage with negligible nitrate content. In previous studies, it could only be speculated that the physiological effects of BR consumption were mediated through the systemic reduction of nitrate-derived nitrite to nitric oxide. In addition to nitrate, BR also contains several other potentially metabolically active compounds that might influence the physiological responses to exercise, including betaine, antioxidants, and polyphenols (10,19,23,30). The use of a nitrate-depleted BR as a placebo in this study enabled us to isolate the effects of dietary nitrate from these other potentially ergogenic components. The unchanged plasma [\text{NO}_3^-], PO, and performance after PL supplementation compared with the presupplementation baseline tests indicates that the acute physiological effects of BR consumption can be attributed, in large part, to its high nitrate content. This does not exclude the possibility, however, that other bioactive compounds in BR might act synergistically with nitrate to positively affect the physiological responses to exercise after longer-term BR supplementation (38).

We have previously reported a reduced VO$_2$ for the same external PO after BR supplementation (1,2,24,38). When other sources of dietary nitrate are not restricted, the steady-state VO$_2$ for a given PO is reduced by approximately 5%–10% (24,38). Conversely, in the present study, this same relationship was evident in the 7%–11% greater PO produced per liter of O$_2$ consumed. Although the mechanism(s) responsible for this effect is/are presently unclear, one possibility is a nitric oxide–mediated improvement in muscle contractile efficiency (1). In a recent study using $^{31}$P magnetic resonance spectroscopy, we observed a reduced total ATP turnover rate and a reduced muscle metabolic perturbation (i.e., a blunting of the exercise-induced fall in [PCr] and increase in [ADP] and [P$_i$]) for the same PO after BR supplementation (1). Whether these changes are consequent to reductions in, for example, the ATP cost of actin–myosin

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interaction or Ca^{2+} handling remains to be determined, but there are suggestions that nitrite/nitric oxide can modulate these processes (14,18,39), as well as the efficiency of mitochondrial respiration (7,25). In addition, nitrate supplementation may enhance bulk muscle blood flow (2,8) and reduce local blood flow-to-VO_2 heterogeneities (5,37), factors that likely also contribute to the enhancement of high-intensity exercise performance. Irrespective of the mechanisms responsible for the apparent ergogenicity of BR, the present results indicate that greater (potential for) nitric oxide production after dietary nitrate supplementation enables a greater PO and hence improved performance during voluntary exercise.

Limitations. To enhance “ecological validity,” we elected to allow subjects to consume their “normal” diet before the first testing session and to reproduce this same diet before all subsequent tests. To facilitate this, the subjects used diaries to record their food and fluid intake and physical activities in the 24 h before the first test session and then consumed the same foods and fluids and completed the same activities in the 24 h preceding the remaining laboratory visits. Compliance to these instructions was good, but on the few occasions where subjects stated that they had not adhered to these guidelines, the tests were rescheduled. In total, four tests had to be rescheduled because of noncompliance during the study: two because of a change in food intake, one because of strenuous exercise the day before testing, and one because of alcohol consumption the evening before testing. On this basis, it would seem reasonable to conclude that the observed differences in blood pressure, the physiological responses to exercise, and TT performance are attributable to the consumption of the nitrate-rich BR. Given the lack of rigid control of diet, however, and the fact that we did not assess factors such as hydration status or sleep quality, we cannot rule out the possibility that nondisclosure of poor adherence to the pretest instructions influenced our results.

CONCLUSIONS

In conclusion, dietary supplementation with a single 0.5-L dose of nitrate-rich BR improved 4- and 16.1-km TT performance in trained cyclists. Plasma [nitrite] was significantly increased 2.5 h after BR supplementation, and systolic blood pressure was reduced, consistent with an increased NO bioavailability. BR ingestion resulted in greater cycling PO with no change in VO_2 such that the PO/VO_2 ratio was significantly increased. The results indicate that acute dietary nitrate supplementation with BR may lead to a significant and practically meaningful enhancement of 4- and 16.1-km TT performance in subelite cyclists.

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

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