No Improvement in Endurance Performance After a Single Dose of Beetroot Juice

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Introduction: Dietary nitrate supplementation has received much attention in the literature due to its proposed ergogenic properties. Recently, the ingestion of a single bolus of nitrate-rich beetroot juice (500 ml, ~6.2 mmol NO₃⁻) was reported to improve subsequent time-trial performance. However, this large volume of ingested beetroot juice does not represent a realistic dietary strategy for athletes to follow in a practical, performance-based setting. Therefore, we investigated the impact of ingesting a single bolus of concentrated nitrate-rich beetroot juice (140 ml, ~8.7 mmol NO₃⁻) on subsequent 1-hr time-trial performance in well-trained cyclists.

Methods: Using a double-blind, repeated-measures crossover design (1-wk washout period), 20 trained male cyclists (26 ± 1 yr, VO₂peak 60 ± 1 ml · kg⁻¹ · min⁻¹, Wₘₚₓ 398 ± 7.7 W) ingested 140 ml of concentrated beetroot juice (8.7 mmol NO₃⁻; BEET) or a placebo (nitrate-depleted beetroot juice; PLAC) with breakfast 2.5 hr before an ~1-hr cycling time trial (1,073 ± 21 kJ). Resting blood samples were collected every 30 min after BEET or PLAC ingestion and immediately after the time trial.

Results: Plasma nitrite concentration was higher in BEET than PLAC before the onset of the time trial (532 ± 32 vs. 271 ± 13 nM, respectively; \( p < .001 \)), but subsequent time-trial performance (65.5 ± 1.1 vs. 65 ± 1.1 s), power output (275 ± 7 vs. 278 ± 7 W), and heart rate (170 ± 2 vs. 170 ± 2 beats/min) did not differ between BEET and PLAC treatments (all \( p > .05 \)).

Conclusion: Ingestion of a single bolus of concentrated (140 ml) beetroot juice (8.7 mmol NO₃⁻) does not improve subsequent 1-hr time-trial performance in well-trained cyclists.

Keywords: nitrate, time trial, cycling, ergogenic aids
compared with a nitrate-depleted beetroot-juice placebo (Lansley, Winyard, Bailey, et al., 2011). Those authors concluded that the ergogenic properties of dietary nitrate ingestion can be applied acutely, making beetroot juice a very effective and practical ergogenic aid with numerous applications in sports practice.

In the current study, we aimed to assess the practical relevance of nitrate-rich beetroot juice as an ergogenic aid in well-trained cyclists. First of all, to obtain a sufficient dose of nitrate, subjects have been required to consume large volumes (500 ml) of beetroot juice (Bailey et al., 2010; Bailey et al., 2009; Lansley, Winyard, Bailey, et al., 2011; Lansley, Winyard, Fulford, et al., 2011; Vanhatalo et al., 2010). Ingesting such a large volume during the last few hours before competition, however, is less practical for athletes when the ingestion of other nutrients may be of greater priority. Therefore, we applied a more concentrated form of beetroot juice providing a similar amount of nitrate (~8.7 mmol NO₃⁻) in a much smaller volume (140 ml). Furthermore, to assess the impact of nitrate-rich beetroot juice in a more practical, competition-simulated environment, we provided a standardized breakfast before the assessment of exercise-performance capacity during a more prolonged time trial (~1 hr). We hypothesized that the ingestion of a single dose of concentrated nitrate-rich beetroot juice would improve subsequent 1-hr time-trial performance in well-trained endurance cyclists. Using a double-blind, crossover design, 20 trained male subjects with an extensive background in either road cycling or triathlon consumed a single dose (140 ml) of concentrated nitrate-rich (~8.7 mmol NO₃⁻) beetroot juice or a nitrate-depleted beetroot-juice placebo in the morning, immediately before ingesting a standardized breakfast. Two and a half hours after consumption of the beetroot juice, endurance-performance capacity was assessed by means of an ~1-hr cycling time trial.

### Methods

#### Subjects

Twenty trained male cyclists or triathletes (26 ± 1 years, 1.83 ± 0.01 m, 74 ± 2 kg) were recruited to participate in the study. All subjects had been engaged in regular cycling training (>3 ×/week) for several years. After being advised of the purpose and potential risks of the study, all subjects provided written informed consent. The experimental protocol and procedures were approved by the medical ethical committee of the Academic Hospital Maastricht, The Netherlands.

#### Study Design

The study was designed to investigate whether ingestion of a single bolus of concentrated beetroot juice (140 ml) improves subsequent ~1-hr time-trial performance. The experimental protocol consisted of four visits to the laboratory, which was maintained at 21 ± 0.5 °C with a relative humidity of 60% ± 5%. All exercise tests were carried out on an electronically braked cycle ergometer (Lode Excalibur, Groningen, The Netherlands). Visit 1 involved an incremental cycling exercise test to exhaustion to determine subjects’ peak oxygen uptake (VO₂peak) and maximal workload capacity (Wmax), and Visit 2 consisted of a familiarization session for the ~1-hr time trial. Visits 3 and 4 consisted of the experimental trials, during which the subjects were given either a single bolus of concentrated nitrate-rich beetroot juice or a nitrate-depleted beetroot juice placebo 2.5 hr before commencing the ~1-hr time trial. The two experimental trials were performed in a double-blind, randomized, counterbalanced order separated by at least 1 week.

#### Wmax and VO₂peak

Subjects’ Wmax (398 ± 7.7 W) and VO₂peak (60 ± 1 ml · kg⁻¹ · min⁻¹) determined using an online gas-collection system (Omnical, Maastricht University, The Netherlands) were assessed during a stepwise exercise test to exhaustion on an electronically braked cycle ergometer (Lode Excalibur, Groningen, The Netherlands). After a 5-min warm-up at 100 W, workload was set at 150 W and increased by 50 W every 2.5 min until voluntary exhaustion (Kuipers, Verstappen, Keizer, Geurten, & van Kraneburg, 1985). Workload, cadence, and heart rate (Polar, Finland) were recorded at every interval. The appropriate seat position and handbar height and orientation were determined and replicated for each subject’s subsequent visit. Wmax was calculated as the last completed stage (W) + time spent in the last incomplete stage (s)/150 (s) × 50 (W).

#### Physical Activity and Dietary Standardization

Subjects kept their weekly training schedule as consistent as possible over the course of the experiment and kept a record of all physical activity performed within 48 hr of each experimental trial. They standardized their workouts 48 hr before each experimental trial and refrained from physical exercise and exhaustive physical labor for 24 hr before each experimental trial. Subjects maintained and recorded their habitual diet for the 48-hr period before the first experimental trial and replicated their diet during the 48 hr before the second trial. They were not restricted in the consumption of nitrate-rich food choices (Lansley, Winyard, Fulford, et al., 2011; Vanhatalo et al., 2010). The evening before each experimental trial, all subjects consumed the same standardized dinner (50 kJ/kg, providing 65 energy% [En%] carbohydrate, 15 En% fat, and 20 En% protein). Furthermore, they all received the same standardized breakfast, consisting of low-nitrate-containing foods (32 kJ/kg, providing 76 En% carbohydrate, 13 En% fat, and 11 En% protein) in the laboratory 2.25 hr before commencing the time trial.

#### Experimental Protocol

For the two main experimental trials, subjects reported to the laboratory in the morning after an overnight fast.
Subsequently, a catheter was inserted into an antecubital vein for venous blood sampling. After a basal blood sample was obtained, subjects consumed 140 ml of concentrated beetroot juice (BEET; 98% concentrated beetroot juice and 2% concentrated lemon juice from Beet It, James White Drinks Ltd., Ipswich, UK) or an equivalent volume of placebo (PLAC) that consisted of nitrate-depleted beetroot juice (James White Drinks Ltd.) as used in previous studies (Cermak et al., 2012; Lansley, Winyard, Bailey, et al., 2011; Lansley, Winyard, Fulford, et al., 2011). For a complete nutrient profile of beetroot, the reader is referred to the USDA database (http://ndb.nal.usda.gov/ndb/foods/list). The nitrate-rich beetroot juice consisted of 8.72 mmol of nitrate per 140 ml. The nitrate concentration of the nitrate-depleted beetroot juice placebo was negligible (0.004 mmol). The nitrate concentration in both the nitrate-rich beetroot juice and nitrate-depleted placebo were measured immediately before the commencement of the study. The nitrate-rich and nitrate-depleted beetroot juice had a similar taste and appearance. To prevent any attenuation in the reduction of nitrate in the oral cavity by commensal bacteria, subjects were asked to refrain from using any antibacterial mouthwash during the 24-hr period before each experimental trial (Govoni, Jansson, Weitzberg, & Lundberg, 2008). Fifteen minutes after consuming the beetroot juice or placebo, subjects consumed the standardized breakfast. After breakfast, and during the 2.25 hr before the time trial, subjects were not allowed to eat or drink except for water. Blood samples were obtained every 30 min for 2.5 hr after the ingestion of the beetroot juice. To simulate a practical, performance-based setting, the blood catheter was removed immediately after the collection of the final resting blood sample (150 min after BEET or PLAC ingestion).

Subjects were subsequently fitted with a heart-rate monitor and positioned on the cycle ergometer. After a 5-min standardized warm-up, they were instructed to perform a set amount of work (1,073 ± 21 kJ) in the shortest time possible. The set amount of work for each participant was based on the $W_{\text{max}}$ capacity determined during Visit 1. Total work to be performed was calculated according to the equation of Jeukendrup et al. (1996): Total amount of work = 0.75 × $W_{\text{max}}$ × 3,600, where $W_{\text{max}}$ is the maximal workload capacity determined during Visit 1 and 3,600 is the duration of the predicted total performance time in seconds (equivalent to 1 hr). The approximately 1-hour exercise duration was selected to invoke a relatively high-intensity (75% $W_{\text{max}}$). Yet non-glycogen-limiting, exercise bout (Jeukendrup, Brouns, Wagenmakers, & Saris, 1997; McConell, Canny, Daddo, Nance, & Snow, 2000). The ergometer was set in linear mode so that 75% $W_{\text{max}}$ was obtained when the subjects cycled at their preferred cycling cadence of 90 rpm. The ergometer was connected to a computer that calculated and displayed the total amount of work performed. Subjects received no temporal, verbal, or physiological feedback during the time trial. The only information available to the subjects was the absolute amount of work performed, which was displayed on a separate computer screen set up in front of the ergometer. A fan was placed 1 m behind each subject to provide cooling and air circulation during the time trials. Water was allowed ad libitum during the first time trial, and the intake was replicated during the subsequent trial. Heart rate, power output, and cadence were recorded continuously throughout the test. All experimental trials were performed at sea level (112 m), which is important because the reduction of nitrite to nitric oxide is potentiated in hypoxia (Vanhatalo et al., 2011). Immediately after the time trial, a single blood sample was taken by venipuncture from an antecubital vein.

**Plasma Analyses**

Blood samples (10 ml) were collected in EDTA-containing tubes and centrifuged at 1,000 g for 10 min at 4 °C. Aliquots of plasma were frozen and stored at −80 °C for subsequent analysis of plasma nitrate ($\text{NO}_3^-$), nitrite ($\text{NO}_2^-$), glucose, insulin, lactate, and free fatty acid concentrations. The plasma nitrate and nitrite concentrations were analyzed by chemiluminescence (Lundberg & Govoni, 2004). Plasma glucose (Uni Kit III, Roche, Basel, Switzerland), lactate (Gutmann & Wahlefeld, 1974), and free fatty acid (NEFA-C, Wako Chemicals, Neuss, Germany) concentrations were analyzed with a COBAS-PENTRA semiautomatic analyzer (Roche). Insulin was analyzed by radioimmunoassay (Linco, human insulin RIA kit, Linco Research Inc., St. Charles, MO).

**Statistical Analyses**

All time-trial performance data were analyzed using a two-tailed paired-sample $t$ test (Sigma Stat 3.1, Point Richmond, CA). Plasma data were analyzed using a two-factor (Treatment × Time) repeated-measures analysis of variance (ANOVA). Linear-regression analysis was performed to examine whether there was a linear relationship with the change in plasma levels of nitrate and/or nitrite immediately before the time trial, with the change in time-trial performance between the BEET and PLAC groups. The level of significance for all analyses was set at $p < .05$, and significant interactions and main effects were subsequently analyzed using Tukey’s post hoc test. All data are presented as $M \pm SE, N = 20$.

**Results**

**Plasma Nitrate and Nitrite Data**

Plasma nitrate concentrations increased from preingestion (0 min) to 120 min after ingestion of BEET compared with PLAC (from 37.7 ± 2.7 to 310 ± 5.3 μM vs. from 37.7 ± 2.8 to 31 ± 2.2 μM; $p < .001$; Figure 1[A]). In line, plasma nitrite concentrations increased from preingestion (0 min) to 120 min postingestion of BEET compared with PLAC (from 238 ± 21 to 532 ± 32 nM vs. from 252 ± 13 to 271 ± 13 nM; $p < .001$; Figure 1[B]).
Plasma Metabolite Data

There were no differences in plasma glucose (Figure 2[A]), insulin (Figure 2[B]), lactate (Figure 2[C]), or free fatty acid (Figure 2[D]) concentrations at rest, post-prandially, and/or after the time trial between the BEET and PLAC trials.

Time-Trial Performance

Time-trial performance was not different between BEET and PLAC (65.5 ± 1.1 vs. 65.0 ± 1.1 min; *p* > .05; Figure 3[A]). Average power output was 275 ± 7 and 278 ± 7 W in the BEET and PLAC trials, respectively, with no differences between trials (p > .05; Figure 3[B]). The average power outputs for every 10% of the time trial completed are presented in Figure 4. No differences were observed in the power output over time between trials. Mean heart rate (170 ± 2 vs. 170 ± 2 beats/min) and mean cycling cadence achieved during the time trial (90 ± 1 vs. 90 ± 1 rpm) were not different between BEET and PLAC (p > .05). The within-subject performance variability between the two time trials was 2.5%. Linear-regression analysis revealed no relationship between the difference in the concentration of nitrate or nitrite immediately before the start of the time trial (120 min) and the difference in time-trial performance between the BEET and PLAC experimental trials (*r*² = .02 and *r*² = .07; *p* > .05).

Discussion

The current study demonstrates that ingestion of a single bolus of concentrated (140 ml) nitrate-rich (8.7 mmol NO₃⁻) beetroot juice does not improve subsequent 1-hr time-trial performance in trained cyclists. Recently, beetroot juice has received much attention in the scientific literature, among athletes and coaches, and in the popular media for its proposed use as an ergogenic aid. Beetroot contains a naturally high concentration of nitrate, which has recently been confirmed as the functional component responsible for the ergogenic properties of beetroot juice (Lansley, Winyard, Fulford, et al., 2011). Exogenous nitrate (NO₃⁻) is reduced to bioactive nitrite (NO₂⁻) by facultative anaerobic bacteria in the saliva and further to nitric oxide (NO) via various pathways (Duncan et al., 1995; Zhang et al., 1998). Although it is well known that NO plays a key role in the regulation of blood flow, muscle contractility, myocyte differentiation, and glucose and calcium homeostasis (Dejam, Hunter, Schechter, & Gladwin, 2004; Webb et al., 2008), there is increasing evidence that improving NO bioavailability through dietary nitrate provision affects metabolic and/or circulatory parameters during exercise (Bailey et al., 2010; Bailey et al., 2009; Lansley, Winyard, Fulford, et al., 2011; Larsen et al., 2007; Larsen, Weitzberg, Lundberg, & Ekblom, 2010; Vanhatalo et al., 2010).
Figure 2 — Plasma concentrations of (A) glucose, (B) insulin, (C) lactate, and (D) free fatty acids (FFA) in the fasted state (0 min), immediately after meal ingestion (30 min), during the postprandial period (60–150 min), and after cessation of the ~1-hr time trial (Post) after ingestion of a single bolus of nitrate-rich beetroot juice (BEET) or a nitrate-depleted beetroot juice (PLAC), $M \pm SE, N = 20$. No differences were observed over time between experimental trials.

Figure 3 — Time-trial (A) performance and (B) mean power output after ingestion of a single bolus of nitrate-rich beetroot juice (BEET) or nitrate-depleted beetroot juice (PLAC), $M \pm SE, N = 20$. No differences were observed between experimental trials.
Whereas previous studies (Bailey et al., 2010; Bailey et al., 2009; Lansley, Winyard, Fulford, et al., 2011) examined the potential ergogenic properties of nitrate-rich beetroot juice after >6 days of supplementation (500 ml, 5.2–11.2 mmol NO$_3^-$/day), recently Lansley, Winyard, Bailey, et al. (2011) observed a significant improvement in athletic performance after the ingestion of only a single bolus (500 ml, ~6.2 mmol NO$_3^-$) of beetroot juice 2.5 hr before 4- and 16.1-km time trials. This opened up the possibility of using beetroot juice as an effective and practical ergogenic aid to be ingested before athletic competition. Therefore, in the current study we examined the ergogenic effects of a single 140-ml bolus of highly concentrated nitrate-rich beetroot juice on subsequent 1-hr time-trial performance in well-trained endurance cyclists. The more practical volume of concentrated beetroot juice (140 ml, ~8.7 mmol NO$_3^-$) yielded plasma nitrite concentrations 120 min after consumption similar to the plasma nitrite concentrations achieved by Lansley, Winyard, Bailey, et al. (532 vs. 575 nM). Despite achieving similar plasma nitrite concentrations however, we were unable to detect any performance benefits after the ingestion of the bolus of nitrate-rich (~8.7 mmol NO$_3^-$) beetroot juice compared with the placebo on subsequent 1-hr cycling performance. Average power output, cycling cadence (rpm), and heart rate did not differ between the nitrate-rich and nitrate-depleted-beetroot-juice trials. Moreover, we did not observe a significant correlation between the changes in plasma NO$_2^-$ concentrations and improvements in time-trial performance. Similar to our previous study (Cermak et al., 2012), we standardized the subjects’ diet between experiments by providing a standardized evening meal the night before each time trial and a standardized breakfast the morning of each trial. To ascertain dietary compliance, subjects consumed breakfast with the investigator in the laboratory during the morning of each time trial. As we achieved plasma nitrite concentrations similar to those of Lansley, Winyard, Bailey, et al., eating breakfast in close proximity with beetroot-juice ingestion does not seem to affect the conversion of NO$_3^-$ to NO$_2^-$. These results imply that athletes may consume a small meal alongside their beetroot-juice ingestion without reducing the capacity to convert NO$_3^-$ to NO$_2^-$. The use of such strict standardized procedures resulted in a coefficient of variation between time trials of less than 2.5%, which is less than the expected day-to-day variability of recreationally trained cyclists (Jeukendrup et al., 1996), with no differences between experimental trials.

Our findings seem to be in contrast to the recent publication of Lansley, Winyard, Bailey, et al. (2011), who reported significant performance improvements in a 4- and 16.1-km time trial after the ingestion of a single 500-ml bolus of beetroot juice (~6.2 mmol NO$_3^-$) 2.5 hr before the onset of exercise. There is no clear explanation for the apparent discrepancy between the two studies, but differences may be attributed to the achieved cycling intensity and/or duration of the time trial that was used in the current study. Previous reports showing performance-enhancing properties of nitrate-rich beetroot juice have measured either indirect (time to exhaustion; Bailey et al., 2010; Bailey et al., 2009; Lansley, Winyard, Fulford, et al., 2011) or direct performance (time trial; Cermak et al., 2012; Lansley, Winyard, Bailey, et al., 2011) over a relatively short exercise duration (6–30 min). In the current study, subjects cycled at 69% ± 1% of their W$_{max}$, requiring 65 ± 1 min to complete the required amount of work. In comparison, Lansley, Winyard, Bailey, et al. had subjects perform short cycling time trials lasting only 6–27 min. Although subjects’ W$_{max}$ capacity was not reported, it is clear that they performed the cycling time trials at a much higher intensity (>69% W$_{max}$) than in the current study. Previously, we observed a small but significant improvement in 10-km time-trial performance after 6 days of dietary nitrate (140 ml/day, ~8 mmol NO$_3^-$/day) supplementation in a group of 12 trained cyclists (Cermak et al., 2012). To complete the 10-km time trial,
subjects performed 15.9 ± 0.29 min of exercise at an intensity of 85% ± 2% \( W_{\text{max}} \). Consequently, it could be speculated that the duration and/or intensity of the performed exercise determines the ergogenic properties of nitrate-rich beetroot juice. Further support for an intensity- or duration-dependent ergogenic effect is found in the recent publication from the Jones group (Wilkerson et al., 2012), who found no significant improvement in 50-mile time-trial performance with the ingestion of a single bolus of beetroot juice. This null finding is in contrast to their shorter, more high-intensity time trials (Lansley, Winyard, Bailey, et al., 2011), further supporting a potential intensity- and/or duration-dependent ergogenic effect of beetroot juice. The reduction of NO\(_2^-\) to NO is potentiated in hypoxic (Castello, David, McClure, Crook, & Poyton, 2006) and acidic (Modin et al., 2001) environments, during which the classic production of NO from the NOS pathway is impaired. As such, in comparison with the shorter, higher intensity time trials, the longer, less intense time trials may limit the development of hypoxic and/or acidic loci in the skeletal muscle and potentially reduce the dependence of NO production through the reduction of NO\(_2^-\) (Wilkerson et al., 2012). The apparent lack of any acute ergogenic effect from beetroot-juice ingestion found in the current study may also be attributed to the duration of the supplementation period and/or total nitrate dose. It has been reported that endurance-trained individuals have higher basal plasma NO\(_3^-\) and/or NO\(_2^-\) concentrations than their untrained counterparts (Bescos et al., 2011; Jungersten, Ambring, Wall, & Wennmalm, 1997; Schena, Cuzzolin, Rossi, Pasetto, & Benoni, 2002), potentially due to a greater training-related NOS activity (McAllister & Laughlin, 2006; McConell et al., 2007). Wilkerson et al. reported ~33% higher resting plasma NO\(_2^-\) than in their previous time-trial study (Lansley, Winyard, Bailey, et al., 2011), which used less well-trained subjects. Thus, perhaps supplementing with a higher dose of NO\(_3^-\) or for a longer period (i.e., >1 day) is necessary to potentiate any ergogenic effect of beetroot juice in well-trained athletes. Due to the relatively novel concept of using nitrate-rich beetroot juice as an ergogenic aid, more research is warranted to determine under which conditions it displays the greater ergogenic properties.

From an athletic viewpoint, although the exact dose and supplementation period required remain unknown, dietary nitrate still represents a functional ergogenic aid when consumed before competition. However, the acceptable daily intake of nitrate as defined by the Joint Expert Committee of the Food and Agriculture Organization of the United Nations/World Health Organization is 3.7/kg body weight (Speijers, 1996), which is equivalent to 259 mg (~4.2 mmol) per day for a 70-kg individual (Alexander et al., 2008) or an ~120-g serving of spinach or beetroot. Currently, research studies demonstrating any performance effect have supplemented subjects with 6–8 mmol NO\(_3^-\)/day (~370–500 mg NO\(_3^-\)/day), which is more than twice the recommended acceptable daily intake. As such, there has been much discussion on the potential impact of high nitrate content in the diet with respect to health and disease (Alexander et al., 2008; Milkowski, Garg, Coughlin, & Bryan, 2010). High levels of dietary nitrate and nitrite have been associated with the production of carcinogenic N-nitrosamines (Tannenbaum & Correa, 1985), which has led to strict regulation of nitrate and nitrite levels in food and drinking water. However, epidemiological data do not provide strong evidence to restrict nitrate consumption (Alexander et al., 2008), and the proposed benefits of nitrate and nitrite in the diet will likely receive much more attention in the near future (Dejam et al., 2004; Tang, Jiang, & Bryan, 2011; Webb et al., 2008). In conclusion, ingestion of a single bolus of concentrated nitrate-rich beetroot juice (8.7 mmol NO\(_3^-\)) does not improve subsequent 1-hr time-trial performance in well-trained cyclists.

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**References**


A possible mediator of ‘acidic-metabolic’ vasodilation.


