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Nitrate Supplementation's Improvement of 10-km Time-Trial Performance in Trained Cyclists

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Six days of dietary nitrate supplementation in the form of beetroot juice (~0.5 L/d) has been reported to reduce pulmonary oxygen uptake (VO_2) during submaximal exercise and increase tolerance of high-intensity work rates, suggesting that nitrate can be a potent ergogenic aid. Limited data are available regarding the effect of nitrate ingestion on athletic performance, and no study has investigated the potential ergogenic effects of a small-volume, concentrated dose of beetroot juice. The authors tested the hypothesis that 6 d of nitrate ingestion would improve time-trial performance in trained cyclists. Using a double-blind, repeated-measures crossover design, 12 male cyclists (31 ± 3 yr, VO_{2peak} = 58 ± 2 ml · kg^{-1} · min^{-1}, maximal power [W_{max}] = 342 ± 10 W) ingested 140 ml/d of concentrated beetroot (~8 mmol/d nitrate) juice (BEET) or a placebo (nitrate-depleted beetroot juice; PLAC) for 6 d, separated by a 14-d washout. After supplementation on Day 6, subjects performed 60 min of submaximal cycling (2 × 30 min at 45% and 65% W_{max}, respectively), followed by a 10-km time trial. Time-trial performance (953 ± 18 vs. 965 ± 18 s, $p < .005$) and power output (294 ± 12 vs. 288 ± 12 W, $p < .05$) improved after BEET compared with PLAC supplementation. Submaximal VO_2 was lower after BEET (45% W_{max} = 1.92 ± 0.06 vs. 2.02 ± 0.09 L/min, 65% W_{max} 2.94 ± 0.12 vs. 3.11 ± 0.12 L/min) than with PLAC (main effect, $p < .05$). Whole-body fuel selection and plasma lactate, glucose, and insulin concentrations did not differ between treatments. Six days of nitrate supplementation reduced VO_2 during submaximal exercise and improved time-trial performance in trained cyclists.

Keywords: beet, beetroot, VO_2, endurance exercise, cycling

Recent work in humans suggests that increasing nitric oxide (NO) bioavailability may induce physiological changes beyond the well-known hemodynamic effects (Dejam, Hunter, Schechter, & Gladwin, 2004; Webb et al., 2008). NO plays a key role in the regulation of blood flow, muscle contractility, myocyte differentiation, and glucose and calcium homeostasis (Dejam et al., 2004). Furthermore, prolonged exposure of mammalian cells to NO has been shown to stimulate mitochondrial biogenesis through cyclic-guanosine monophosphate-dependent pathways (Clementi & Nisoli, 2005; Nisoli et al., 2004). In the human body, exogenous nitrate (NO_3^-) is reduced to bioactive nitrite (NO_2^-) by facultative anaerobic bacteria in the saliva and further to NO via various pathways (Duncan et al., 1995; Zhang et al., 1998). Recently, several groups (Bailey et al., 2010; Bailey et al., 2009; Lansley, Winyard, Fulford, et al., 2011; Larsen, Weitzberg, Lundberg, & Ekblom, 2007, 2010; Vanhatalo et al., 2010) investigated whether dietary nitrate provision affects metabolic or circulatory parameters during exercise in vivo in humans. Oral ingestion of sodium nitrate (0.1 mmol · kg^{-1} · day^{-1}) for 2–3 days has been shown to significantly reduce pulmonary oxygen uptake (VO_2) during submaximal cycling exercise in both untrained (Larsen et al., 2010) and trained men (Larsen et al., 2007). Because the use of sodium nitrate is regulated in most countries, researchers have started to examine the impact of ingesting nitrate-rich foods such as beetroot juice on the physiological response to exercise (Bailey et al., 2010; Bailey et al., 2009; Lansley, Winyard, Bailey, et al., 2011; Lansley, Winyard, Fulford, et al., 2011; Vanhatalo et al., 2010).

Jones and colleagues have demonstrated that ingesting 0.5 L of beetroot juice per day for 6 days reduces VO_2 during submaximal exercise (Bailey et al., 2010; Bailey et al., 2009; Lansley, Winyard, Fulford, et al., 2011; Vanhatalo et al., 2010) and lowers the ATP cost of muscle-force production, suggesting enhanced contractile efficiency (Bailey et al., 2010). This improvement in exercise efficiency was evident acutely (2.5 hr) after ingestion of a single 0.5-L bolus of beetroot juice and persisted for 15 days when supplementation was continued (Vanhatalo et al., 2010). Although the demonstrated cardiovascular and physiological benefits of beetroot juice have been assumed to be the result of its high nitrate concentration, beetroot juice is rich in several other potentially metabolically active compounds (e.g., polyphenols). To confirm nitrate as the active component for the proposed benefits, Jones and colleagues (Lansley, Winyard, Fulford, et al., 2011) tested beetroot juice (~6.2 mmol NO_3^-) against nitrate-depleted (~0.0047 mmol NO_3^-) beetroot juice. They verified their previous findings by demonstrating a lowered O_2 cost of submaximal exercise after 6 days of supplementation with nitrate-rich beetroot juice (0.5

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L) compared with nitrate-depleted beetroot juice. These results suggest that further work using beetroot juice should focus on nitrate as the mediator for any ergogenic benefits.

From the proposed improvements in metabolic efficiency, Jones and colleagues (Bailey et al., 2010; Bailey et al., 2009) have demonstrated that 6 days of nitrate supplementation may increase exercise tolerance or time to fatigue when exercise is performed at higher work rates. These results suggest that inorganic nitrate may act as a strong ergogenic aid, but studies testing any performance effects of nitrate are limited (Bescos et al., 2011; Lansley, Winyard, Bailey, et al., 2011) and often not designed in a practical, performance-based manner (Bescos et al., 2011). The latter is also in reference to the large doses (0.5 L) of beetroot juice that have been applied in former studies (Bailey et al., 2010; Bailey et al., 2009; Lansley, Winyard, Bailey, et al., 2011; Lansley, Winyard, Fulford, et al., 2011; Vanhatalo et al., 2010), which may be impractical for athletes to consume before competition. As such, no study has investigated the potential ergogenic properties of nitrate and its effects on whole-body fuel selection after 6 days of concentrated (0.14 L) beetroot-juice supplementation using a practical, performance-based test that closely simulates athletic competition in a trained population. We hypothesized that 6 days of ingestion of concentrated nitrate-rich beetroot juice (0.14 L) would reduce VO_2 during submaximal exercise and improve time-trial performance in endurance-trained cyclists. In a double-blind, crossover design, 12 trained male subjects with an extensive background in either road cycling or triathlon were subjected to 6 days of concentrated nitrate or placebo (nitrate-depleted beetroot juice) supplementation. VO_2 and whole-body fuel selection were assessed during 60 min of submaximal exercise at work rates corresponding with 45% and 65% maximal power (W_{max}), respectively. Thereafter, performance capacity was assessed by means of a 10-km simulated time trial.

Methods

Subjects

Thirteen trained male cyclists or triathletes (31 ± 3 years, 177 ± 1.7 cm, 73 ± 2 kg) were selected to participate in this study, but one did not comply with the study protocol and was excluded. All subjects were engaged in regular cycling training (~10 hr/week) and had a training history of ~10 years. Subjects' W_{max} (342 ± 10 W) and VO_{2peak} (58 ± 2 ml · kg^{-1} · min^{-1}) capacity, determined using an online gas-collection system (Expair, Medisoft Group, Lille, France), were assessed during a step-wise exercise test to exhaustion on the subject's own bicycle attached to a Computrainer (Racermate, Seattle, WA). To incorporate a warm-up into the step-wise test to exhaustion, subjects began pedaling at 100 W for 1 min, then continued at 135 W for 2 min and 165 W for 2 min. Thereafter, work rate was increased by 35 W every 3 min until exhaustion. W_{max} was calculated as the last completed stage (W) + time in last stage (s)/180 × 35 (W). After being advised of the purpose and potential risks of the study, all subjects provided written, informed consent. The experimental protocol was approved by the Hamilton Health Sciences/Faculty of Health Sciences research ethics board.

Experimental Protocol

The study was designed to investigate whether 6 days of concentrated dietary nitrate supplementation (0.14 L) lowers VO_2 and modulates whole-body fuel selection during relatively low- (45% W_{max}) and moderate- (65% W_{max}) intensity exercise or improves 10-km time-trial performance. Subjects visited the laboratory 2 weeks before the experimental trials to perform a familiarization trial that was identical to the main experimental protocol, consisting of two 30-min bouts of cycling at 45% (155 ± 4.4 W) and 65% (224 ± 6.3 W) W_{max}, immediately followed by a 10-km time trial. For the main experiment, subjects consumed 2 × 70 ml (~8 mmol NO_3^-/day) of concentrated beetroot juice (Beet It, James White Drinks Ltd., Ipswich, UK) or an equivalent amount of placebo (nitrate-depleted beetroot juice, Beet It; Lansley, Winyard, Fulford, et al., 2011) every morning after breakfast for 6 days before testing. During the morning of the final day of supplementation, subjects reported to the laboratory 2.5 hr after consuming a standardized breakfast and their last supplemental beverage. They rested for 10 min before their blood pressure was taken four times using an automated cuff (SunTech Medical, Morrisville, NC), with the last three measurements averaged to obtain mean blood pressure. Subsequently, a catheter was inserted into an antecubital vein for venous blood sampling. After a resting blood sample was obtained, subjects initiated a 60-min continuous endurance-exercise session consisting of 30 min at 45% W_{max} and 30 min at 65% W_{max} performed on the subject's own bicycle attached to a Computrainer (Racermate Inc., Seattle, WA) calibrated 0, 15, 30, and 60 min into the exercise bout. Cardiorespiratory data were collected for 5–8 min at 5, 20, 35, and 50 min into the exercise bout, and values of VO_2, VCO_2, respiratory-exchange ratio, and heart rate were averaged over the last 3 min of each sampling period. Ratings of perceived exertion using the Borg 6–20 scale were recorded after cardiorespiratory measurements. Rates of whole-body carbohydrate and fat oxidation were calculated based on the equations published by Péronnet and Massicotte (1991). Immediately after the 60-min submaximal ride, subjects performed a simulated 10-km cycling time trial on the Computrainer as described previously (van Essen & Gibala, 2006). In short, the subject's rear bicycle wheel was attached to the Computrainer, which was connected to a laptop computer displaying the 10-km flat course. Power output and speed were recorded by the provided software, and water was allowed ad libitum. Subjects received no temporal, verbal, or physiological feedback during the time trial and were only aware of

the distance they had covered. Blood samples were obtained every 15 min at 0, 15, 30, 45, and 60 min during the submaximal exercise bout and again immediately after completion of the time trial (~75–80 min after the start of submaximal exercise). To simulate a practical, performance-based setting, no blood samples or cardiorespiratory measurements were taken during the time trial. The two experimental trials (after nitrate or placebo supplementation) were performed at the same time of day in a randomized and double-blind fashion and separated by a 14-day washout period. Exercise tests were performed at sea level (237 m), which is important because the reduction of nitrite to nitric oxide is potentiated in hypoxia (Vanhatalo et al., 2011)

Physical Activity and Dietary Standardization

Subjects kept their weekly training schedule as consistent as possible over the course of the experiment. All subjects standardized their workouts performed during each 6-day intervention period (nitrate and placebo) but were not allowed to exercise 24 hr before each testing day. They maintained their habitual diet over the course of the study and 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 experiment, all subjects consumed the same standardized dinner (52 kJ/kg, providing 61 energy% [En%] carbohydrate, 19 En% fat, and 20 En% protein). Furthermore, all subjects received the same standardized breakfast (39 kJ/kg, providing 75 En% carbohydrate, 13 En% fat, and 12 En% protein) 2.5 hr before reporting to the laboratory for testing. 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 course of the study (Govoni, Jansson, Weitzberg, & Lundberg, 2008) and to refrain from alcohol and caffeine for 24 hr before testing.

Plasma Analyses

Blood samples (8 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 –20 °C for subsequent analysis of plasma nitrate (NO_3^-), glucose, insulin, lactate, and free-fatty-acid concentrations. Plasma nitrate was analyzed spectrophotometrically according to the Griess reaction (Miranda, Espey, & Wink, 2001). Plasma glucose (Uni Kit III, Roche, Basel, Switzerland) and lactate (Gutmann & Wahlefeld, 1974) concentrations were analyzed with a Cobas-Pentra semiautomatic analyzer (Roche), and free-fatty-acid (NEFA-C; Wako Chemicals, Neuss, Germany) concentration was analyzed with a Cobas-Fara semiautomatic analyzer (Roche). Insulin was analyzed by radio immunoassay (Linco, human insulin RIA kit, Linco Research Inc., St. Charles, MO).

Statistical Analyses

All time-trial performance and blood-pressure data were analyzed using a two-tailed paired *t* test (Sigma Stat 3.1, Point Richmond, CA). To determine the time-trial effect size, Cohen's *d* statistical calculation was used and achieved power was calculated. Cardiorespiratory and plasma data were analyzed using a two-factor (Treatment × Time) repeated-measures analysis of variance (ANOVA). 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 $M \pm SE$, $N = 12$.

Results

Blood Pressure

Resting systolic (119 ± 3 vs. 119 ± 3 mm Hg) and diastolic (74 ± 2 vs. 73 ± 2 mm Hg) blood pressure did not differ between treatments ($p > .05$) after 6 days of supplementation.

Cardiorespiratory Data

Mean VO_2 was lower at 45% and 65% W_{max} with beetroot juice than with placebo (Figure 1). There were no differences between treatments in VCO_2, heart rate, respiratory-exchange ratio, or ratings of perceived exertion (Table 1). In line, there were no differences between treatments in whole-body carbohydrate-oxidation rates during submaximal exercise, but there was a trend for fat-oxidation rates to be lower with the beetroot juice than with placebo ($p = .08$; Table 1). Both whole-body carbohydrate-oxidation and fat-oxidation rates were higher at 65% than at 45% W_{max} (carbohydrate oxidation 40.8 ± 1.4 vs. 26.2 ± 1.1 kJ/min, fat oxidation 22.9 ± 1.7 vs. 15.3 ± 1.2 kJ/min, respectively; $p < .001$). There were no differences between treatments in total energy expenditure (Table 1).

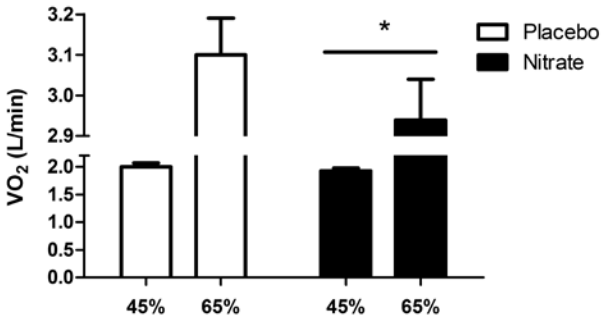

Figure 1 — Mean pulmonary oxygen uptake (VO_2) during submaximal cycling exercise at 45% and 65% maximal power (W_{max}) after 6 days of supplementation with either nitrate or a nitrate-depleted placebo, $M \pm SE$, $N = 12$. *$p < .05$ vs. placebo.

Table 1 Cardiorespiratory Data During Submaximal Exercise, *M* ± *SE*, *N* = 12

	Placebo		Nitrate	
Variable	**45% W_{max}**	**65% W_{max}**	**45% W_{max}**	**65% W_{max}**
VO_2 (L/min)	2.0 ± 0.07	3.1 ± 0.09	1.93 ± 0.05*	2.94 ± 0.10*
VCO_2 (L/min)	1.74 ± 0.06	2.69 ± 0.08	1.71 ± 0.04	2.63 ± 0.08
Respiratory-exchange ratio	0.87 ± 0.01	0.88 ± 0.01	0.89 ± 0.01	0.89 ± 0.01
Carbohydrate oxidation (kJ/min)	24.9 ± 1.2	38.8 ± 2.0	28.2 ± 2.0	42.8 ± 1.9
Fat oxidation (kJ/min)	17.2 ± 1.7	26.0 ± 2.2	13.3 ± 1.7	19.8 ± 2.3
Total energy (kJ)	1261 ± 51	1945 ± 63	1227 ± 30	1875 ± 66
Heart rate (beats/min)	121 ± 3	148 ± 4	121 ± 3	147 ± 3
Rating of perceived exertion	10.5 ± 0	14.2 ± 0	10.2 ± 0	14.1 ± 0

Note. All measurements were performed after a standardized breakfast ingested 2.5 hr before the onset of exercise.

*$p < .05$ vs. placebo.

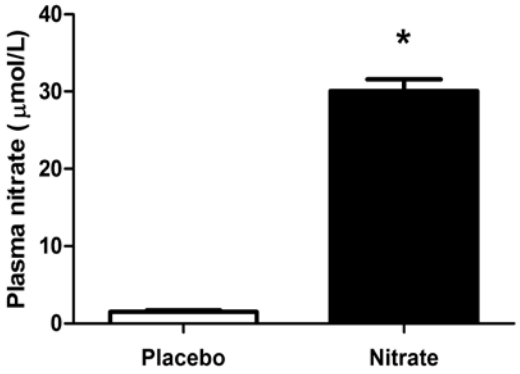

Figure 2 — Plasma nitrate concentration after 6 days of supplementation with either nitrate or a nitrate-depleted placebo, *M* ± *SE*, *N* = 12. *$p < .05$ vs. placebo.

Plasma

Plasma nitrate concentration assessed after each 6-day supplementation regimen showed higher values after beetroot juice than placebo (30.1 ± 1.5 vs. 1.5 ± 0.2 µmol, $p < .05$; Figure 2). There were no differences between treatments in plasma glucose (Figure 3[A]), insulin (Figure 3[B]), or lactate (Figure 3[C]) concentrations at rest, during submaximal exercise, and after the time trial. Plasma free-fatty-acid concentrations measured immediately after cessation of the time trial were higher in the beetroot juice than the placebo treatment (373 ± 57 vs. 286 ± 35 µmol/L, $p < .05$; Figure 3[D]).

Time-Trial Performance

Time to complete the 10-km time trial was lower (1.2%) with beetroot juice than with placebo (953 ± 21 vs. 965 ± 21 s, $p < .005$; Figure 4[A]), and this was associated with a higher (2.1%) mean power output (294 ± 12 vs. 288 ± 12 W, $p < .05$; Figure 4[B]). Mean heart rate during the time trial was not different between treatments (171 ± 3 vs. 169 ± 4 beats/min, respectively, $p > .05$).

The within-subject day-to-day variability in time-trial performance was 1%. The calculated Cohen's *d* effect size was small (.2), but the achieved statistical power for the time-trial performance was .98.

Discussion

The current study demonstrates that 6 days of concentrated dietary nitrate supplementation (~8 mmol/day NO_3^-) lowers VO_2 during submaximal exercise and improves 10-km time-trial performance in trained endurance cyclists. Previous work has demonstrated that 3–15 days of dietary nitrate supplementation lowers mean VO_2 during submaximal exercise (Bailey et al., 2010; Bailey et al., 2009; Lansley, Winyard, Fulford, et al., 2011; Larsen et al., 2007; Vanhatalo et al., 2010). We confirmed these results by demonstrating that 6 days of nitrate supplementation using concentrated beetroot juice (0.14 L/day) lowers VO_2 by 3.5% and 5.1% at work rates corresponding to 45% and 65% W_{max}, respectively, in trained cyclists.

The reduction in VO_2 is surprising because classical exercise physiology dictates minimal fluctuations in oxygen consumption for any individual at any given work rate, regardless of training status, age, or diet (Moseley, Achten, Martin, & Jeukendrup, 2004). The precise mechanisms by which nitrate administration lowers VO_2 during exercise have not been elucidated. However, the mechanism responsible for a lower VO_2 during exercise is likely related to the role of NO_2^- and NO as regulators of cellular O_2 utilization (Clementi & Nisoli, 2005; Clerc, Rigoulet, Leverve, & Fontaine, 2007; Dejam et al., 2004; Larsen et al., 2011; Zhang et al., 1998). Although NO_2^- was not measured in the current study, NO_2^- can be

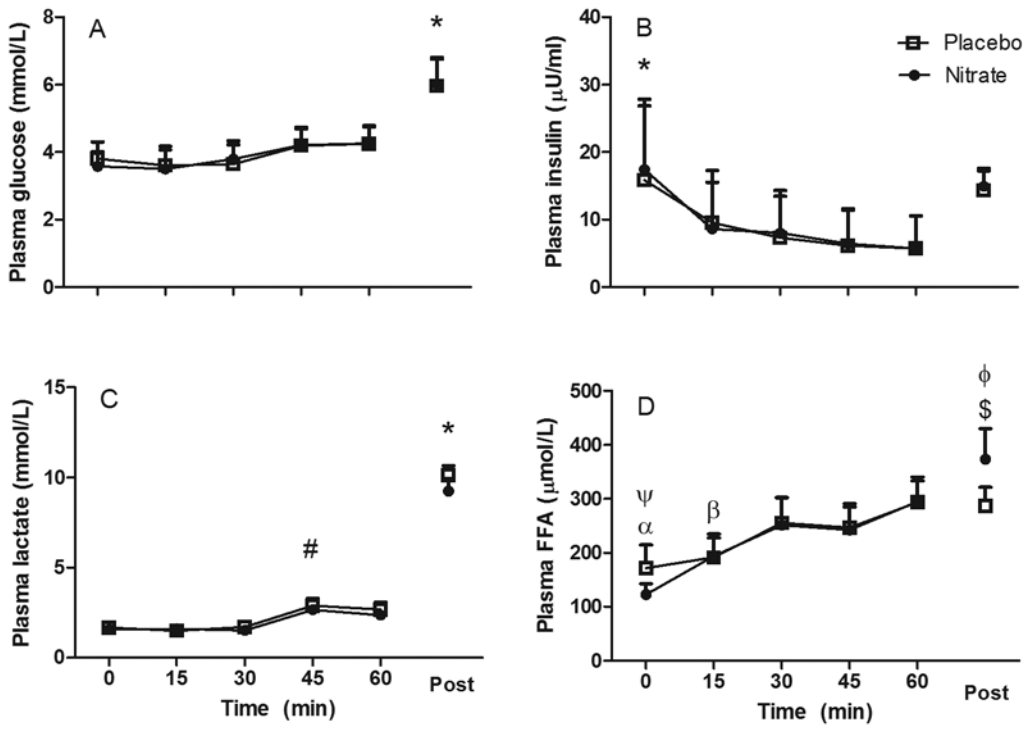

Figure 3 — Concentrations of (A) glucose, (B) insulin, (C) lactate, and (D) free fatty acids (FFA) at rest (0 min), during 30 min of submaximal cycling exercise at 45% maximal power (15–30 min), during 30 min of submaximal cycling exercise at 65% maximal power (45–60 min), and after cessation of the 10-km time trial (Post) after 6 days of supplementation with either nitrate or a nitrate-depleted placebo, $M \pm SE$, $N = 12$. $\$p < .05$ vs. placebo Post. $^{*}p < .05$ vs. all other time points. $\#p < .05$ vs. 0, 15, and 30 min. $^{\phi}p < .05$ vs. 0, 15, 30, and 45 min. $^{\alpha}p < .05$ vs. 30, 45, and 60 min. $^{\beta}p < .05$ vs. 60 min. $^{\psi}p < .05$ vs. 30 and 60 min and Post.

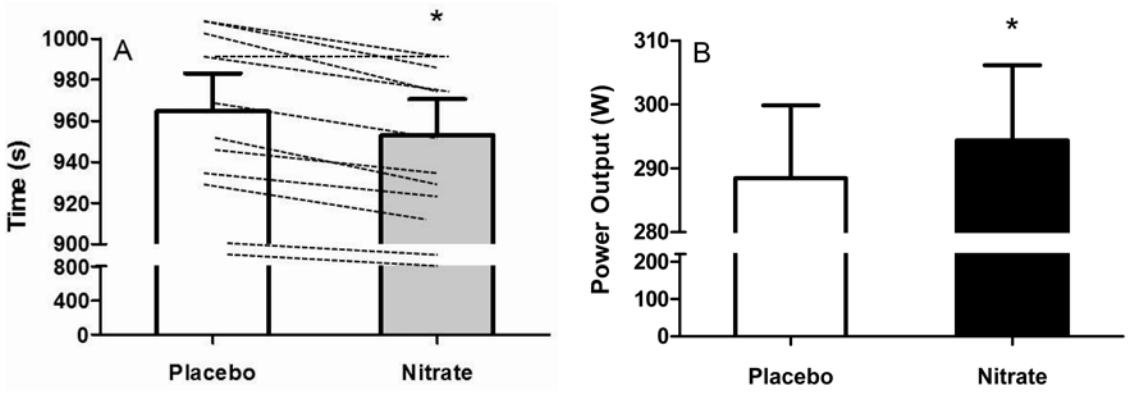

Figure 4 — 10-km time-trial performance with (A) individual data and (B) mean power output after 6 days of supplementation with either nitrate or nitrate-depleted placebo, $M \pm SE$, $N = 12$. $^{*}p < .05$ vs. placebo.

reduced to NO (Nohl et al., 2000) by accepting reducing equivalents from Complex III of the electron-transport chain (Kozlov, Staniek, & Nohl, 1999) and is known to be an important inhibitor of cytochrome c oxidase activity (Brown & Cooper, 1994). It has been suggested that NO may enhance the efficiency of oxidative phosphorylation by reducing "slippage" of the mitochondrial proton pumps or attenuating the expression of uncoupling proteins (Clerc et al., 2007), reducing the total ATP cost of muscle-force production (Bailey et al., 2010), or some combination of the three, thereby improving metabolic efficiency. In an elegant study, Larsen et al. (2011) recently assessed basal mitochondrial function after 3 days of dietary nitrate supplementation and revealed a tighter coupling between respiration and oxidative phosphorylation in skeletal-muscle tissue. They also reported

a higher P:O ratio after nitrate supplementation, which corresponds with a greater amount of ATP produced per oxygen consumed. Other work has also indirectly demonstrated that nitrate supplementation lowers the total ATP cost of muscle-force production (Bailey et al., 2010), rather than an improvement in a higher P:O ratio. Regardless of the precise mechanisms, the current study is in line with both those findings (Bailey et al., 2010; Larsen et al., 2011) and previous work (Nohl et al., 2000; Shen, Tian, Saupe, Spindler, & Ingwall, 2001), supporting the contention that nitrate supplementation improves skeletal-muscle mitochondrial efficiency, even in trained athletes.

Greater exercise efficiency after nitrate supplementation might translate into superior performance capacity. In agreement, previous studies have reported a longer time to exhaustion during exercise after 4–6 days of dietary nitrate supplementation (Bailey et al., 2010; Bailey et al., 2009; Lansley, Winyard, Fulford, et al., 2011). However, such time-to-exhaustion protocols do not necessarily imply improvements in exercise performance in a more practical setting that simulates normal athletic competition. Therefore, to assess the potential ergogenic effects in a more practical manner after the established 6-day supplementation protocol (Bailey et al., 2010; Bailey et al., 2009; Lansley, Winyard, Fulford, et al., 2011), we studied the impact of 6 days of concentrated dietary nitrate supplementation on time-trial performance after 1 hr of submaximal cycling exercise in trained cyclists. Performance improvements were observed in 11 of 12 cyclists, clearly revealing the ergogenic properties of nitrate supplementation on time-trial performance.

The minimum dose and duration of nitrate supplementation needed to elicit ergogenic effects remain largely unknown. Recently, Lansley, Winyard, and Bailey (2011) adopted the more practical time-trial design to investigate the acute ergogenic effects of nitrate after only one dose (~6.2 mmol NO_3^-) ingested 2.5 hr before completion of a 4- and 16.1-km time trial. Performance improved in both distances after nitrate ingestion, suggesting that the ergogenic effects of nitrate are acutely attainable. However, in the former study, subjects were required to ingest 0.5 L of beetroot juice, which may be impractical for athletes to ingest in the last few hours preceding an athletic event. Therefore, the current study demonstrated for the first time that administering a similar dose of nitrate (8 mmol) through concentrated beetroot juice (0.14 L), thereby ingesting a much smaller volume, is an equally effective ergogenic aid. Both studies complement each other by demonstrating that the ergogenic benefits of nitrate supplementation are evident during both short (~6.3 min; Lansley, Winyard, & Bailey, 2011) and longer exercise bouts (~75 min). After nitrate ingestion, subjects improved 2.8% and 2.7% in the 4- and 16.1-km time trials, respectively (Lansley, Winyard, & Bailey, 2011), and in the current study, subjects improved 1.2% in their 10-km time-trial performance. The smaller improvements found in the current study may be, at least partly, attributed to small differences in the performance level of the cyclists. Furthermore, it could be speculated that the ergogenic properties of nitrate ingestion are more pronounced during shorter exercise bouts because the current study used a 1-hr submaximal exercise protocol before commencing the time trial. More research will likely follow to address the optimal feeding strategies to maximize performance.

With dietary nitrate administration representing a functional ergogenic aid, there will be much speculation on the impact of acute and long-term nitrate supplementation on overall health. Currently, the acceptable Daily Intake of nitrate is 3.7 mg · kg body weight^{-1} · day^{-1}, which is equivalent to 222 mg (~3.6 mmol) per day for a 60-kg individual (Alexander et al., 2008). The latter would translate to an ~120-g serving of spinach or beetroot. In contrast, subjects were supplemented in the current study with 8 mmol nitrate per day (~500 mg/day). To obtain this amount of nitrate through nitrate-containing whole foods, this would translate to consuming 200–300 g of spinach or beetroot (Lundberg & Govoni, 2004). The latter would be roughly 2 to 4 times the average recommended serving size (~85 g) and more than twice the recommended Acceptable Daily Intake. Therefore, to achieve an ergogenic effect, nitrate ingested as sodium nitrate or via a carrier such as concentrated beetroot juice provides an attractive and feasible alternative to ingesting copious amounts of whole nitrate-rich foods. There has been much discussion, however, of 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, dietary nitrate supplementation lowers mean VO_2 values during submaximal exercise and improves 10-km time-trial performance in trained cyclists. These results suggest that nitrate or food products naturally high in nitrate can be used as an effective ergogenic aid for competitive athletes.

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