Dietary Nitrate Supplementation Improves Rowing Performance in Well-Trained Rowers

Hannah Bond, Lillian Morton, and Andrea J. Braakhuis

Increased plasma nitrate concentrations from dietary sources of nitrate have proven to benefit exercise performance. Beetroot (BR) contains relatively high levels of nitrate (NO_3^-), which increases nitric oxide stores. This study investigated whether dietary nitrate supplementation, in the form of a BR beverage, would improve rowing performance during ergometer repetitions. In a randomized crossover design, 14 well-trained junior male rowers consumed 500 ml of either BR or placebo (PL) daily for 6 d. After supplementation, rowers completed 6 maximal 500-m ergometer repetitions and times were recorded. A 7-d washout period separated the 2 trials. Blood pressure, oxygen saturation, maximum heart rate, urine (specific gravity, pH, and nitrites), and lactates were collected for analysis at baseline and pre- and postperformance. Changes in the mean with 95% confidence limits were calculated. There was a likely benefit to average repetition time in the BR condition, compared with PL (0.4%, 95% confidence limits, ± 1.0%). In particular, Repetitions 4–6 showed an almost certain benefit in rowing time on BR (1.7%, 95% CL, ± 1.0%). The underlying mechanism for the observed results remains unknown, as differences observed in rowers' physiological measures between the 2 conditions were unclear. Conclusively, nitrate supplementation in the form of BR juice resulted in improved maximal rowing-ergometer repetitions, particularly in the later stages of exercise.

Keywords: vegetables, maximal exercise, repeated efforts, nitric oxide

Nitric oxide (NO) is a widespread signaling molecule and has a number of physiological roles in regulating organ functions but is known primarily for maintaining normal blood pressure and protecting the cardiovascular system (Willmot, Gray, Gibson, Murphy, & Bath, 2005; Zand, Lanza, Garg, & Bryan, 2011). Under the appropriate physiological conditions, nitrate (NO_3^-) and nitrite (NO_2^-) are recycled to form NO and complement the NO synthase-dependent pathway where NO is produced via the oxidation of L-arginine (Lundberg, Weitzberg, & Gladwin, 2008). Dietary NO_3^- (from green leafy vegetables and beetroot) is reduced to NO_2^- by nitrate reductase (Zand et al., 2011), causing a sustained increase in circulating NO_2^- levels (Lundberg & Govoni, 2004). Plasma NO_2^- is further reduced to the bioactive NO (Larsen, Weitzberg, Lundberg, & Ekblom, 2010). This nitrate-nitrite-NO pathway has been shown in both human and animal trials to reduce blood pressure (Larsen et al., 2010; Kapil[AUQ1] et al., 2010) and improve oxygen utilization, particularly during tissue acidosis and hypoxia, when oxygen tension falls (Bescós et al., 2011[AUQ2]; Lundberg et al., 2008).

Dietary NO_3^- has 100% bioavailability (Jeukendrup & Gleeson, 2004) and is readily absorbed in the upper gastrointestinal tract, with approximately 25% of ingested NO_3^- taken up by and excreted by the salivary glands (Zand et al., 2011). Dietary nitrate and nitrite can therefore be considered storage pools for NO bioactivity (Lundberg et al., 2008). Dietary supplementation of foods high in nitrates increases the concentration of plasma nitrite levels. Larsen, Weitzberg, Lundberg, and Ekblom (2007) reported plasma levels of 182 ± 55 μM in their nitrate group after 3 days of supplementation (0.1 mmol sodium nitrate · kg^{-1} · day^{-1}) compared with 27 ± 6.9 μM in the placebo group. Likewise, Larsen et al. (2010) found that plasma nitrite concentrations increased at rest after nitrate supplementation of 0.033 mmol $NaNO_3$/kg body weight three times daily for 2 days (230 ± 31 μM compared with placebo 17.3 ± 3.0 μM), and Bailey et al. (2009) showed that plasma nitrite concentrations increased on average by 96% after 6 days supplementation of 5.5 mmol/day NO_3^-. Even an acute dose 2 hr before exercise of 500 ml of beetroot juice has been shown to result in a 138% increase in plasma NO_2^- relative to placebo (Lansley et al., 2011[AUQ3]). Therefore, dietary nitrate in the form of 500 ml of beet juice has proven to increase nitrite concentrations. The ability to increase NO bioavailability through supplementing with foods containing adequate amounts of nitrate, such as beetroot juice, is useful given the ease of digestion and availability of the juice.

Research examining the effects of dietary nitrate supplementation on exercise performance in humans has shown promising results. Larsen et al. (2007) tested nine young, well-trained men on maximal and submaximal cycling exercise tests after 3 days of dietary nitrate

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supplementation (0.1 mmol $NaNO_3 \cdot kg^{-1} \cdot day^{-1}$). The study showed that the oxygen cost of exercise at submaximal work rates was markedly reduced. Gross muscle efficiency increased from 19.7% ± 1.6% during placebo to 21.1% ± 1.3% during nitrate supplementation. Larsen et al. (2010) found similar results. After 2 days of nitrate supplementation (0.033 mmol $NaNO_3$/kg body weight three times daily), subjects performed incremental exercise tests to exhaustion with two combined arm and leg ergometers. After nitrate supplementation, VO_2 was reduced from 3.72 ± 0.33 L/min during placebo to 3.62 ± 0.31 L/min during nitrate supplementation ($p < .05$). Despite a reduction in VO_2, time to exhaustion increased after nitrate supplementation (563 ± 30 s) compared with placebo (524 ± 31 s). Similar findings were reported in a study by Bailey et al. (2009), where supplementation of 500 ml of beetroot juice for 6 consecutive days resulted in significantly reduced O_2 cost of cycling at fixed submaximal work rates and increased time to exhaustion during severe-intensity exercise. Reduced VO_2 is typically coupled with a decrease in work performance (Larsen et al., 2010), yet nitrate supplementation results in increased time to exhaustion. It appears that only a moderate dose of dietary nitrate (0.033 mmol $NaNO_3$/kg three times daily) may be for performance improvements by possibly improving the energy efficiency of working muscles.

A common finding in the nitrate studies examining exercise performance is that heart rate, lactate concentration, CO_2 production, minute ventilation, and respiratory-exchange ratio all show no significant change between nitrate and placebo groups. A reduction in blood pressure has been observed after nitrate supplementation, as the nitrate-nitrite-NO pathway is important in the regulation of blood pressure, blood flow, and vasodilatation (Bescós et al., 2011[AUQ4]; Zand et al., 2011). Larsen et al. (2007) found that resting systolic blood pressure was reduced after nitrate supplementation (112 ± 8 mm Hg) compared with placebo (120 ± 5.9 mm Hg), as was resting diastolic blood pressure (BR = 120 ± 5.9 mm Hg vs. PL = 74 ± 6.8 mm Hg). Bailey et al. (2009) found that ingestion of beetroot juice significantly reduced systolic blood pressure by an average of 6 mm Hg relative to placebo, but diastolic blood pressure and average arterial pressure were not significantly different after beetroot ingestion. These results are similar to those found by Lansley et al. (2011)[AUQ5], where systolic blood pressure was reduced significantly relative to placebo (BR = 125 ± 5 vs. PL = 131 ± 8 mm Hg), but diastolic blood pressure and mean arterial pressure were not significantly altered by beetroot ingestion.

Research has demonstrated that dietary nitrate supplementation reduces the O_2 cost of submaximal cycling and intense cycling and improves performance in maximal combined arm and leg exercise (Larsen et al., 2010). An acute dose of 500 ml of beetroot juice has also resulted in reduced performance times in a 16.1-km cycling time trial relative to placebo (Lansley et al., 2011[AUQ6]). It appears that exercise requiring maximal oxygen utilization, such as endurance exercise, shows the greatest performance gains from nitrate supplementation. This is because these exercise zones require the presence of oxygen due to increased oxygen consumption in active muscles (Larsen et al., 2007). However, to date there is no literature on the effects of dietary nitrate on elite rowers and rowing performance.

Due to the highly competitive nature of the sport of rowing, there is always pressure for optimal performance, especially on the world stage. Providing insight into this line of research may provide an opportunity for athletes to implement a new nutritional strategy that improves performance with a very low risk of consuming a supplement that might contain a banned substance. The aim of this study was to investigate whether dietary supplementation with beetroot juice, containing high amounts of nitrates, improves repeated rowing-ergometer performance. We hypothesized that after nitrate ingestion in the form of beetroot juice, rowing performance time would be reduced.

Methods

Subjects

Fourteen well-trained junior male rowers (*M* ± *SD* age 16.7 ± 0.5 years, weight 82.8 ± 6.6 kg, height 1.88 ± 0.04 m) volunteered for this study. Rowers were completing 4 hr of training a day. All subjects were healthy and injury free with at least one season's rowing experience. They were informed of the risks and potential benefits associated with the study. Informed written consent was provided by all subjects before the commencement of the study. The proposed procedures in this study were approved by the Wintec Ethics Committee.

Study Procedures

Subjects were randomly assigned in a double-blind crossover design to one of two conditions: beetroot (BR) or placebo (PL). The BR was homemade beetroot juice (5.5 mmol/day of NO_3^-) and PL was a commercially available black-currant juice (with negligible nitrate content). The content of nitrate in the BR beverages was calculated using average beetroot nitrate concentrations and the quantity of beetroot added to the drink. The energy content of both beverages was isocaloric. Subjects were unaware of the experimental hypothesis and were informed that the purpose of the study was to compare physiological responses after supplementation of two commercially available beverages on rowing-ergometer performance. They were familiarized with the testing protocol before data collection. Baseline ergometer times were collected over a period of 1 month (which equated to four 6 × 500-m ergometer sessions). A 6-day supplementation period was implemented where subjects were required to consume 500 ml of their designated beverage each day (250 ml in the morning, 250 ml in the afternoon). On Day 6 of the supplementation period, subjects were required to arrive at the gymnasium in a rested state in preparation for exercise testing. They performed a light warm-up on a rowing ergometer (Concept 2, New Zealand) before testing. After warm-up, subjects completed 6 × 500-m rowing-ergometer repetitions at maximal intensity, with a recovery period of approximately 90 s between repetitions. Six repetitions were used as the rowers used this protocol in their training. Rowing time was recorded to the closest millisecond. All testing was carried out at the same time of day, in the same location, and in the same order. The coach was present at all sessions, reviewing rowers' attendance and work ethic. After testing, subjects had a 7-day washout period and then consumed the other beverage. After the supplementation period, subjects returned to the gymnasium and testing procedures were repeated.

Physiological Measures

One day before the commencement of supplementation, baseline data were collected for urine measuring specific gravity, pH, and nitrites (Intect 7, Branon Medical Corp., USA), as well as preliminary measures for blood pressure (mm Hg, Trimline sphygmomanometer, PyMaH Corp., USA) and oxygen saturation (%, pulse oximeter, Vacumed, USA). On the day of testing, subjects reported to the gym, where further urine samples, blood pressure, and oxygen saturation were measured. After subjects completed all six rowing efforts, blood lactate (mmol/L; Lactate Pro Analyzer, Arkray Inc., Japan), heart rate (beats/min; Polar FS1, Finland), and oxygen saturation were measured. Oxygen-saturation and blood-pressure data were taken again at 1 min and 2 min postexercise.

Food Diaries and Dietary Control

Subjects were provided with a list of foods high in nitrates and were asked to abstain from consuming these foods throughout the duration of the study. On the day of testing, they provided a 24-hr food recall indicating all foods, drinks, and supplements consumed in the last 24 hr. Food diaries were analyzed in FoodWorks 2009 (Version 6, Xyris Software, Australia), which calculated average total energy, carbohydrate, and protein intakes.

Statistics

A postintervention-only crossover spreadsheet (Hopkins, 2006) was used to compare BR and PL. Baseline ergometer times were used as a covariate and 0.2% for the smallest important or harmful effect. All performance variables were log-transformed to express findings as percentage change. For inferences to be made about the population values of the effect of nitrate supplementation on rowing performance, the uncertainty in the effect was expressed as 95% confidence limits. Likelihoods were expressed that the true value of the effect represents substantial benefit or harm (Hopkins, 2006). Possible mechanisms and dietary analysis were reported as $M \pm SD$ at 90% confidence limits.

Results

Across all repetitions, performance time in the BR condition was likely improved compared with PL (0.4%, 95% CL, ± 1.0). Mean performance times for each repetition are shown in Figure 1. Analysis of rowing-ergometer performance in Repetitions 1–3 revealed a possibly negative effect of BR supplementation (1.0%, 95% CL ± 1.7), while Repetitions 4–6 showed an almost certain benefit in rowing time with BR supplementation (1.7%, 95% CL ± 1.0; Table 1). Diastolic blood pressure at 1 min postexercise demonstrated a nearly significant change in the BR group compared with PL (p = .0564) Urine nitrate concentrations were 2.50 ± 1.2 mg/dl after BR supplementation, which was not significantly different than PL (2.43 ± 0.9 mg/dL). No clear differences were found in maximal heart rate, oxygen saturation, lactate accumulation, or urine pH (Table 3). Dietary analysis of the subjects' 24-hr food recall revealed no significant differences in total energy, carbohydrate, or protein intake between the two conditions. Total energy consumption in the BR condition was 15,530 ± 3,822 kJ and in PLA was 15,399 ± 5,376 kJ, while carbohydrate intakes in the BR and PLA groups were 490 ± 150 and 490 ± 200 g, respectively (Table 2).

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Discussion

The principal finding of this study was that supplementation of 500 ml of BR juice for a period of 6 days resulted in improved repeated high-intensity rowing-ergometer performance times. Performance was improved in the BR condition by 0.4% across all repetitions, and in the later stages of exercise (Repetitions 4–6) by 1.7%. Previous studies have reported performance improvements after nitrate supplementation at submaximal workloads, constant-work-rate exercise (Bailey et al., 2009; Larsen et al., 2007), and in cycling time-trial performance (Lansley et al., 2011[AUQ7]). The current study provides insight into the effects of nitrate supplementation on high-intensity exercise with repeated efforts, and results indicate that this type of exercise is enhanced with nitrate supplementation. The 0.4% increase observed across all repetitions would be practically valuable to rowers, as the smallest worthwhile enhancement for top-ranked rowing finalists has been found to be ~0.3% (Smith & Hopkins, 2011). In addition to this, the 1.7% improvement in Repetitions 4–6 indicates that nitrate supplementation has the potential to benefit rowers' performance in the later stages of performance.

It is known that the nitrate-nitrite-NO pathway is activated under higher metabolic demand and hypoxic and acidic conditions (Lundberg et al., 2008). In the later stages of testing, these conditions would have been more evident, and NO could be derived by nitrite oxidation.

Nitrate supplementation has previously been shown to reduce the ATP cost of muscle-force production (Bailey et al., 2009) and increase the capacity for ATP synthesis in mitochondria by enhancing nitrate-coupling efficiency (Larsen et al., 2011). Examination of isolated muscle mitochondria after nitrate supplementation has found that proton leak was reduced, and the amount of oxygen reduced per ATP produced (P:O ratio) was increased (Bescós et al., 2011[AUQ8]; Larsen et al., 2011; Nair, Irving, & Lanza, 2011; Rolfe, Newman, Buckingham, Clark, & Brand, 1999). This improves overall mitochondrial efficiency, thereby reducing whole-body oxygen cost during exercise. It is unclear whether the performance improvements observed in the current study can be attributed to improved muscle-force production or mitochondrial coupling, as power output and metabolic mechanisms were not measured. Future studies examining nitrate supplementation and rowing performance should include a measurement of power output, to assess mechanical efficiency, and VO_2, to assess oxygen cost.

The observed beneficial effect of BR supplementation on rowing-ergometer performance occurred without any significant differences in the physiological measures investigated. Maximal heart rate, oxygen saturation, and lactate accumulation after 6 × 500-m rowing-ergometer repetitions were not significantly different between BR and PLA. Significant changes in both systolic and diastolic blood pressure after nitrate supplementation have been observed in previous studies, even after acute doses in the form of nitrate-rich BR juice (Webb et al., 2008). Contrary to previous findings, no significant differences in systolic blood pressure were evident between the two conditions at any stage throughout testing procedures in the current study. Diastolic blood pressure did, however, demonstrate a nearly significant change in the BR group compared with PL (p = .0564) at 1 min postexercise. Nitrate is known as a potent vasodilator (Larsen et al., 2010), and it is hypothesized that nitrite-induced vasodilation may be responsible for the effect on blood pressure seen after exercise in the BR group. It is hypothesized that this vasodilation results in improved blood flow to working muscles (Bailey et al., 2009). If this were the case one would expect a change in lactate, maximal heart rate, and oxygen saturation, yet these variables did not change significantly, indicating that changes to oxygen delivery are not responsible for the performance gains seen with nitrate supplementation. These findings provide support for the theory that it is improved mitochondrial efficiency that may be responsible for performance improvements (Nair et al., 2011).

The assessment of nitrate in urine may have affected assessment of nitrate values after supplementation. Increases in plasma nitrite have been observed after nitrate supplementation in a number of studies, some by as much as 138% (Lansley et al., 2011[AUQ9]). The nitrite values in this study were not significantly different between the two groups (BR = 2.50 ± 1.2 mg/dl vs. PL = 2.43 ± 0.9 mg/dl).

The performance improvements in the current study were not due to familiarization. The subjects were involved in the same training program with regard to the frequency and intensity of training throughout the study period. The testing protocol implemented in the study is one that the subjects performed once every 2 weeks as part of their own training for that particular training phase. Furthermore, it is apparent that the performance enhancements were not due to increased carbohydrate (BR = 490 ± 153 g vs. PL 486 ± 196 g) or overall energy intake (BR = 15,530 ± 3,822 kJ vs. PL = 15,399 ± 5,376 kJ) based on the 24-hr food recalls. We can only assume that the subjects adhered to the dietary regimen required in the current study. It is difficult to determine whether the subjects' levels of fatigue contributed to the overall results. The fact that the athletes were reaching their peak in preparation for their major regatta of that season may have resulted in greater performance benefits being seen as a result of reaching their training peak.

In conclusion, nitrate supplementation using 500 ml of BR juice for 6 days demonstrated a clear improvement in maximal repeated 6 × 500-m rowing-ergometer bouts. BR supplementation appears to provide greater benefit in the later stages of performance and improved rowing performance times in repeated high-intensity efforts. The BR juice was well tolerated by the subjects, and food-based supplements with true ergogenic potential in as little as 6 days are worthy of adoption into elite athletes' nutritional regimen. There is little to no risk of a positive result in a drug test, which elite athletes are fearful of, and BR juice adds additional vegetable serves to the diet. Although the physiological mechanisms behind the performance effects of BR juice are still unclear, the results of this study indicate that 500 ml of BR juice per day for 6 days may result in improved performance, particularly in repeated high-intensity efforts. Whether these results would be found in a competition setting or a race situation of 2,000 m is yet to be established, but an improvement in training output should result in improved competition performance.

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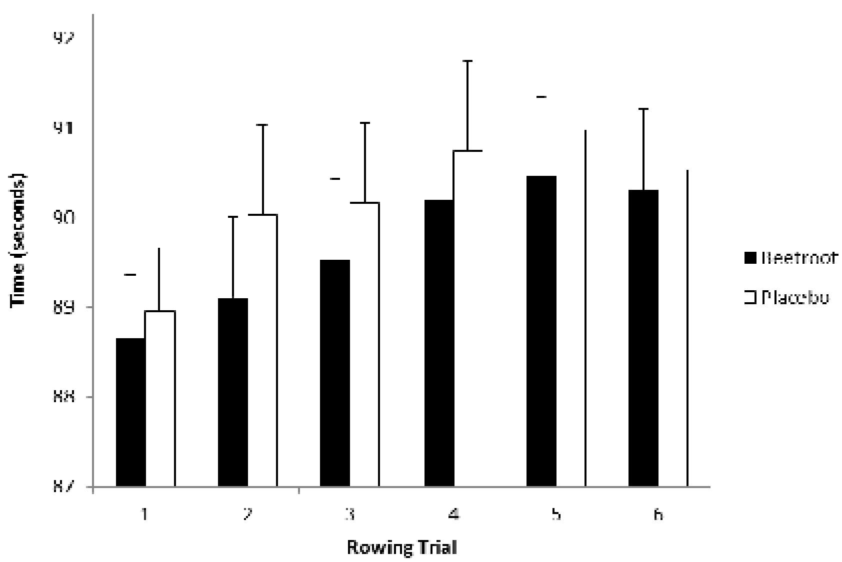

Figure 1 — Mean (± *SE*) rowing-performance times (s) for 500-m rowing-ergometer efforts after beetroot and placebo supplementation.

Table 1 Rowing Speed After Supplementation With Beetroot Juice and Placebo

Repetition #	Baseline mean (s)	Beetroot mean (s)	Placebo mean (s)	Effect on performance (%)	± 95% CL
1–3	90.09	89.09	89.41	–1.0	1.7
4–6	91.02	90.29	90.09	1.7	1.0
1–6	90.53	89.40	90.19	0.4	1.0

Note. CL = confidence limit.

Table 2 Energy, Carbohydrate (CHO), and Protein Analysis of 24-hr Food Recall

	BR (*M* ± *SD*)	PL (*M* ± *SD*)	± 95% CL
Total energy, MJ	15.5 ± 3.8	15.4 ± 5.4	2.4
CHO, g	490.1 ± 150	485.5 ± 200	83.9
Protein, g	179.7 ± 60	177.6 ± 70	36.1

Note. BR = beetroot; PL = placebo; CL = confidence limit.

Table 3 Physiological Measures for Beetroot (BR) and Placebo (PL)

Physiological variable	BR (*M* ± *SD*)	PL (*M* ± *SD*)	± 95% CL
Urine			
pH	6.18 ± 1.9	5.29 ± 0.7	1.2
nitrites (mg/dl)	2.50 ± 1.2	2.43 ± 0.9	0.7
specific gravity	1.01 ± 0.0	1.01 ± 0.0	0.0
Oxygen saturation, %			
0 s postexercise	96.86 ± 1.4	96.93 ± 1.3	1.1
1 min postexercise	96.93 ± 1.1	96.93 ± 1.1	1.1
2 min postexercise	96.86 ± 1.1	96.86 ± 0.9	1.1
Blood lactate, mmol/L			
postexercise	14.8 ± 1.4	14.5 ± 1.0	0.9
Diastolic blood pressure, mm Hg			
0 s postexercise	72 ± 8	69 ± 10	7.6
1 min postexercise	70 ± 7	63 ± 10	7.4
2 min post exercise	67 ± 8	65 ± 9	6.8
Systolic blood pressure, mm Hg			
0 s postexercise	184 ± 20	175 ± 21	10.1
1 min postexercise	166 ± 25	160 ± 25	14.1
2 min postexercise	152 ± 21	148 ± 22	11.7

Note. CL = confidence limit.

Author Queries

[AUQ1] Kapil et al. not in the reference list. Please correct the citation, add the reference to the list, or delete the citation.

[AUQ2] Should this be 2010 or 2011?

[AUQ3] The in-text citation "Lansley et al., 2011" is not in the reference list. Please correct the citation, add the reference to the list, or delete the citation.

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