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The Effects of a Single Dose of Concentrated Beetroot Juice on Performance in Trained Flatwater Kayakers

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The aim of the current study was to determine the effects of dietary nitrate ingestion on parameters of sub-maximal and supramaximal exercise and time trial (TT) performance in trained kayakers. Eight male kayakers completed four exercise trials consisting of an initial discontinuous graded exercise test to exhaustion and three performance trials using a kayak ergometer. The performance trials were composed of 15 min of paddling at 60% of maximum work rate, five 10-s all-out sprints, and a 1 km TT. The second and third trials were preceded by ingestion of either 70 ml nitrate-rich concentrated beetroot juice (BR) or tomato juice (placebo [PLA]) 3 hr before exercise using a randomized crossover design. Plasma nitrate (PLA: $33.8 \pm 1.9 \mu\text{M}$, BR: $152 \pm 3.5 \mu\text{M}$) and nitrite (PLA: 519.8 ± 25.8 , BR: $687.9 \pm 20 \text{ nM}$) were higher following ingestion of BR compared with PLA (both $p < .001$). VO_2 during steady-state exercise was lower in the BR trial than in the PLA trial ($p = .010$). There was no difference in either peak power in the sprints ($p = .590$) or TT performance between conditions (PLA: $277 \pm 5 \text{ s}$, BR: $276 \pm 5 \text{ s}$, $p = .539$). Despite a reduction in VO_2 , BR ingestion appears to have no effect on repeated supramaximal sprint or 1 km TT kayaking performance. A smaller elevation in plasma nitrite following a single dose of nitrate and the individual variability in this response may partly account for these findings.

Dietary supplementation with either sodium nitrate or nitrate-rich beetroot juice (BR) has been consistently shown to reduce resting blood-pressure (Kapil et al., 2010; Vanhatalo et al., 2010; Webb et al., 2008) and the oxygen demand of submaximal exercise in healthy individuals (Bailey et al., 2009, 2010; Lansley et al., 2011a; Larsen et al., 2007; Vanhatalo et al., 2010). Furthermore, ingestion of BR has been reported to improve exercise time to exhaustion during both running (Lansley et al., 2011a) and cycling (Bailey et al., 2009) and enhance cycling and rowing performance (Bond et al., 2012; Cermak et al., 2012a; Lansley et al., 2011b). The reported improvement in performance ranges typically from 1.2–2.9%, and although this change appears small in some instances, it has been suggested that a difference of more than 0.6% for an elite athlete is substantial enough to be of benefit (Paton & Hopkins, 2006). In contrast, recent studies using highly trained participants found no

improvement in performance following ingestion of BR (Cermak et al., 2012b; Wilkerson et al., 2012).

Although the physiological mechanism(s) underlying the reduction in oxygen cost and subsequent enhancement of exercise performance are unclear at present, it is possible that these effects were mediated by a nitrate induced increase in circulating nitrite leading to an elevation in bioactive nitric oxide (NO) and consequent improvement in muscle contractile efficiency (Bailey et al., 2010) or in the efficiency of mitochondrial respiration (Larsen et al., 2011). Elevation of circulating nitrite can be obtained via the ingestion of leafy green and root vegetables that are rich in nitrate and can be used for NO production through the breakdown of nitrate to nitrite in the mouth by bacterial nitrate reductases (Lundberg & Weitzberg, 2010). The nitrite is subsequently converted spontaneously to bioactive NO and following this process NO may increase muscle blood flow, glucose uptake, and cellular respiration and regulate muscular contraction (Stamler & Meissner, 2001).

Research to date has primarily focused on the effects of dietary nitrate on performance during submaximal running and cycling exercise. However, there is also evidence suggesting that nitrate supplementation may enhance tolerance to severe and maximal intensity exercise (Bailey et al., 2009; Larsen et al., 2010; Vanhatalo et al., 2011), high-intensity exercise performance (Lansley et al., 2011b), maximal force production of isolated rat type II muscle fibers (Hernández et al., 2012) and repeated

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maximal rowing sprint performance (Bond et al., 2012). The mechanism underpinning these effects may be the attenuated muscle metabolic perturbation and reduced rate of PCr depletion following nitrate supplementation (Bailey et al., 2010). Given that PCr is essential for brief high-intensity work or repeated supramaximal sprints, this mechanism supports the notion that nitrate supplementation may, in theory, enhance maximal or repeated supramaximal bouts of exercise. Despite this, there has been very limited research conducted to explore this hypothesis.

Of further interest is that only one study has employed an exercise modality where there is a significant contribution from the upper-body (Bond et al., 2012). Although BR ingestion was shown to enhance rowing performance, the authors did not measure the oxygen cost of exercise or indeed the difference in plasma nitrate and nitrite between conditions. However, previous studies have shown that upper-body exercise is less economical at a given submaximal power output (PO) than lower-body exercise (Sawka, 1986) and therefore the effects of nitrate supplementation on the physiological responses to this exercise modality is worthy of further investigation. Paddle sports such as kayaking predominantly involve dynamic muscular actions of the upper-body, however, isometric contractions of muscles in the legs and in the torso are also important for stabilization (Sawka, 1986). Kayaking is an Olympic event held on flat water and competed over varying distances from sprint events (200 m) to middle distance (1 km). The aerobic contribution during a 1 km kayaking performance test has been determined to be 85% (Tesch et al., 1976; Zamparo et al., 1999) and although anaerobic capacity is important for performance there is a greater demand on the aerobic system. During race conditions kayakers spend the majority of the race utilizing their peak oxygen consumption ($\text{VO}_{2\text{peak}}$; Bishop, 2000; Zamparo et al., 1999) and at this intensity kayakers produce 57% of the PO and 87–89% of the $\text{VO}_{2\text{peak}}$ compared with that of cyclists.

No study has yet examined the effects of BR ingestion on the oxygen cost of submaximal upper-body exercise or on kayaking performance. Therefore the aim of this study was to investigate the effects of dietary nitrate supplementation on the oxygen cost, repeated sprint performance, and time trial (TT) performance of trained kayakers. We hypothesized that an acute dose of dietary nitrate would reduce the oxygen cost of submaximal kayaking exercise and improve repeated sprint and 1 km TT kayaking performance.

Methods

Subjects

Eight trained male kayakers (age 31 ± 15 years, stature 182 ± 3 cm, body mass 81.2 ± 8.7 kg, and $\text{VO}_{2\text{peak}}$ 49.0 ± 6.1 mL·kg⁻¹·min⁻¹) volunteered and provided written informed consent to participate in the study (approved

by the Faculty of Science, Engineering and Computing Ethics Committee, Kingston University). Each participant was recruited from local paddle sport clubs and was classed as trained as they regularly completed kayak training and took part in competitive races. All procedures were conducted in accordance with the Declaration of Helsinki.

Experimental Design

Each participant visited the laboratory on four separate occasions. On their first visit they each completed a maximal discontinuous graded kayaking test for determination of $\text{VO}_{2\text{peak}}$ and maximum work rate (WR_{max}). Following this, each participant completed three performance trials at the same time of day, at least five days apart. The first performance trial consisted of a baseline measurement of performance and the remaining two trials were preceded by ingestion of either BR (70 ml of concentrated nitrate rich BR with a listed nitrate content of ~5 mmol, [Beet IT organic shot, James White Drinks Ltd, Ipswich]) or placebo (PLA; 70 ml tomato juice with a listed nitrate content of ~0.01 mmol, [Schweppes, Coca Cola, London]) 3 hr before the start of exercise in a randomized cross-over design (Kenjale et al., 2011). Pharmacokinetic data suggest that plasma nitrite will peak 2.5–3 hr after ingestion of a single dose of BR (Webb et al., 2008) and this method of nitrate delivery has been demonstrated to enhance exercise performance (Lansley et al., 2011b). Furthermore, Fulford et al. (2013) have recently reported that the increase in plasma nitrite is similar whether a single dose was ingested 2.5 hr before exercise or supplementation continued for 5 or 15 days. Although a nitrate-depleted version of BR (Beet IT, James White Drinks Ltd, Ipswich) is now available as a PLA for research purposes, this was not available for commercial sale at the time the study commenced. It was therefore not possible to blind participants to the specific supplementation given the distinctive taste of the drinks. Nevertheless, participants were not aware of the true purpose of the study and were simply informed that the researchers wished to compare the effects of two different antioxidant vegetable/fruit based drinks. The supplements were provided in bottles with no markings to remove manufacturer's statements on the packaging relating to the ergogenic effects of BR. The researchers who conducted the exercise tests were not involved in the dissemination of supplements to participants and participants were asked not to reveal which supplement they had ingested to these individuals. A similar method of delivering the PLA has been employed by several other BR supplementation studies (Bailey et al., 2009; Kenjale et al., 2011; Vanhatalo et al., 2010). Participants were asked to follow their normal diet and activity patterns, although they were requested not to exercise or consume alcohol for 24 hr before each test, not to consume caffeine for 6 hr or to consume anything other than water in the 3 hr before testing.

Experimental Procedures

Maximal Exercise Test

Following standard anthropometric measurements, $\text{VO}_{2\text{peak}}$ and WR_{max} were measured using a discontinuous graded exercise test on a kayak ergometer (Dansprint ApS, Denmark) with a drag resistance coefficient of 27. Pilot data collected in our laboratory in a small sample of kayakers revealed this to be the optimum drag resistance to recreate on water kayaking in our sample population and the discontinuous design of the test resulted in a higher $\text{VO}_{2\text{peak}}$ than a continuous incremental protocol. Participants initially paddled at 50 W for 5 min after which the work rate increased by 25 W every 2 min until volitional exhaustion or the required paddle rate could no longer be maintained. The increase in work rate was interspersed with a 30 s rest period between each 2-min stage of the exercise test. Throughout the test PO and stroke rate were continuously recorded via the Dansprint analyzer (v1.12, Denmark), heart rate was measured by telemetry (Polar Electro, Oy, Finland) and breath by breath respiratory variables were measured and recorded via indirect calorimetry (Oxycon Pro, Jaeger, Germany).

Performance Trials

One week after the maximal exercise test, each participant completed three separate kayak specific performance trials using an adapted version of the protocol described by Bishop et al. (2003). This consisted of 15 min of continuous, steady-state paddling at 60% of WR_{max} followed immediately by five 10-s maximal effort sprints, with each sprint separated by 50 s of active recovery (light paddling). Following a 5-min rest period participants completed a 1 km TT. During this section of the trial the participant was instructed to paddle at a freely chosen velocity and encouraged to complete the 1 km in the quickest time possible. Verbal encouragement was given throughout the TT and participants received visual feedback from a computer monitor only on the distance completed. Heart rate, PO, and respiratory variables were continuously monitored throughout each trial as previously described. The data collected during submaximal exercise is presented as the average for each 3 min period, and data for the 1 km TT was time aligned and averaged for every 100 m completed.

Blood Collection and Analysis

Before the start of each performance trial participants were required to remain in the supine position for 10 min after which blood pressure was measured using an automated blood pressure monitor (Omron M4 Digital BP, Omron Healthcare, Wegelaan, Netherlands) and 4 ml of venous blood was collected from the cephalic vein. The blood was collected in a tube containing EDTA and immediately centrifuged at 4,000 rpm at 4 °C for 10 min (Bailey et al., 2009). The plasma was then separated into two cryovials and immediately frozen in liquid nitrogen

before being stored at –80 °C for a maximum of 4 months for later analysis of nitrate and nitrite via ozone-based chemiluminescence (Rogers et al., 2005).

Nitrite Assay. Triiodide reagent (70 ml Glacial acetic acid, 650 mg iodine, 20 ml HPLC grade water and 1 g Potassium iodide) was placed into a glass purge vessel covered with a rubber septum injection inlet before being heated in a water bath to 50 °C and nitrogen gas added. The purge vessel was subsequently linked to a trap containing 40 ml of 1 M sodium hydroxide solution that was further connected to an NO analyzer (Sievers NOA 280i, Analytix, UK).

A standard curve was produced by injecting 100 μL of nitrite solutions (1000 nM, 500 nM, 250 nM, 125, 62.5 nM), and one sample of HPLC grade water. The latter was injected to account for the nitrite in the water that was added when creating the solutions. Following this, blood plasma samples were thawed in a water bath at 37 °C for 3 min and 100 μL of the sample was injected into the purge vessel and the concentration of NO was determined. The area under the curve was calculated using Origin software (version 7) and divided by the gradient of the slope obtained from the standard curve to determine the levels of nitrite in each sample. The nitrite assay has been shown to be sensitive to $< 10 \text{ nmol}\cdot\text{L}^{-1}$ with a coefficient of variation (CoV) of 5% (Peacock et al., 2012).

Nitrate Assay. Vanadium reagent (785 mg Vanadium chloride, 80 ml 1M Hydrochloric acid, 20 ml HPLC grade water) was injected into a glass purge vessel, and covered with a rubber septum injection inlet while being heated in a water bath maintained at 80 °C.

A standard curve was produced by injecting 15 μL of nitrate solutions (100 μM , 50 μM , 25 μM , 12.5 μM , 6.25 μM) and one sample of HPLC water. Following this blood plasma samples were thawed as before and 15 mL of the sample was injected into the purge vessel. The nitrate levels were calculated as previously described for the nitrite assay. The nitrate assay has been shown to be sensitive to $< 1 \mu\text{mol}\cdot\text{L}^{-1}$ with a CoV of 7% (Peacock et al., 2012).

Data Analysis

Data are reported as mean and standard error of the mean (mean $\pm$ SEM). Differences in blood pressure, plasma nitrate and nitrite, mean PO and TT completion time between PLA and BR conditions were assessed using a paired samples *t* test. The remaining data were analyzed using a two-factor, within-subjects, repeated-measures ANOVA to examine the effects of supplement (BR or PLA), time, and the interaction between the two. Post hoc analysis was completed using Bonferroni multiple comparisons. The test-retest reliability of the steady-state VO_2 measurements was assessed by comparing data from baseline and PLA trials using a number of statistical methods: 1) the mean difference in VO_2 between trials and 95% confidence intervals (95% CI), 2) the single measure intraclass correlation coefficient (ICC) and 95%

CI, and 3) Bland and Altman (1986) analysis to assess 95% limits of agreement (LOA; i.e., mean difference $\pm 1.96 SD_{diff}$). Experimental procedures were identical for these two trials with the exception of the ingestion of 70ml of tomato juice 3 hr before exercise in the PLA trial. The null hypothesis was rejected when $p < .05$. 95% CI are included together with P values, where appropriate. All statistical procedures were completed using SPSS for Mac version 19.0.

Results

Plasma Nitrate and Nitrite and Blood Pressure

The concentration of plasma nitrate in the baseline trial (35 ± 2 mM) did not change following PLA (33 ± 2 μ M, $p = .809$), but did increase significantly after BR ingestion (152 ± 4 μ M, $p < .001$, 95% CI 116–233 μ M; Figure 1). Plasma nitrite also increased after BR compared with baseline ($p < .001$, 95% CI 106–130 nM) but was not affected by PLA ($p = .707$; Baseline: 513 ± 26 nM, PLA: 520 ± 26 nM, BR: 688 ± 20 nM; Figure 2). There was a significant negative correlation between the baseline plasma nitrite concentration and the change in plasma nitrite concentration following BR ingestion ($R = -0.871$, $p = .005$; Figure 3). There were no differences in either systolic (PLA: 132 ± 3 mmHg, BR: 130 ± 2 mmHg, $p = .570$) or diastolic blood pressure (PLA: 73 ± 3 mmHg, BR: 72 ± 3 mmHg, $p = .560$) between trials.

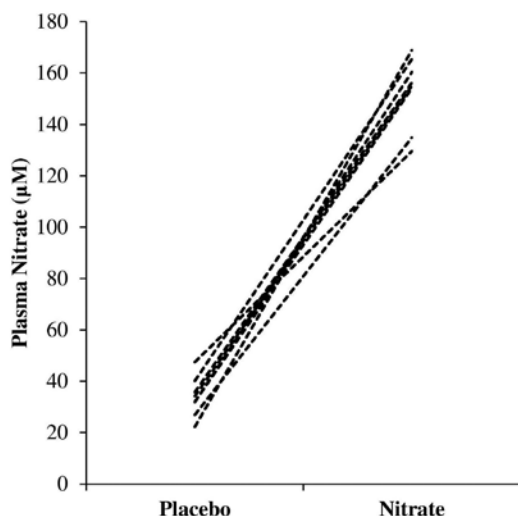


Figure 1 — Plasma nitrate concentrations 3 hr post supplementation during PLA and BR trials. Data are presented as individual responses (dashed lines). Plasma nitrate significantly increased following BR supplementation (PLA: 33 ± 2 , BR: 152 ± 4 μ M, $p < .001$).

15-Min Submaximal Exercise

There was no difference in mean PO between PLA and BR trials throughout the steady-state period (PLA: 108 ± 22 W, BR: 108 ± 23 W, $p = .428$). HR at the end of the 15 min of submaximal steady-state exercise was slightly reduced following BR supplementation (PLA: 158 ± 25 , BR: 156 ± 21 beats·min⁻¹) but was not statistically different between trials ($p = .082$). VO_2 was significantly lower in the BR trial compared with PLA with a mean

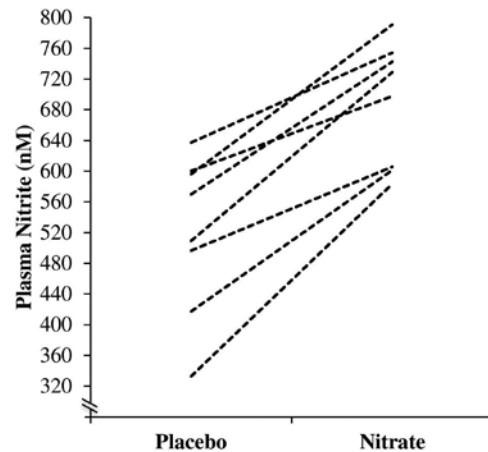


Figure 2 — Plasma nitrite concentrations 3 hr post supplementation during PLA and BR trials. Data are presented as individual responses (dashed lines). Plasma nitrite significantly increased following BR supplementation (PLA: 520 ± 26 , BR: 688 ± 20 nM, $p < .001$).

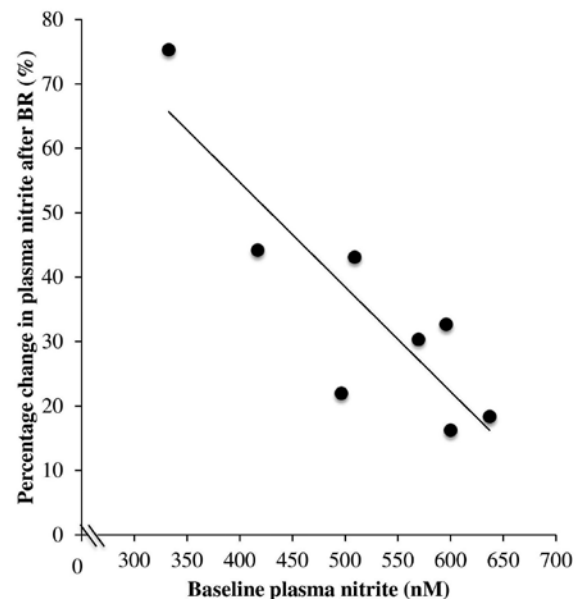


Figure 3 — Relationship between individual baseline plasma nitrite concentrations and the percentage change in plasma nitrite after BR supplementation ($R = -0.871$, $p = .005$).

reduction of 3.1% ($p = .010$, Figure 4). Post hoc analysis revealed that VO_2 was significantly reduced at the 6-, 12-, and 15-min intervals (all $p < .01$). VO_2 measured at the 15-min interval was $36.8 \pm 2.4 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ in the PLA trial and $35.6 \pm 2.5 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ in the BR trial.

Test-Retest Reliability of VO_2

There was no difference in VO_2 measured during the steady-state period between baseline and PLA trials ($p = .29$, mean difference $0.3 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ (0.9%), 95% CI $-0.9 - 0.3 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ (-2.6 - 0.9%), 95% LOA $-1.1 - 1.7 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ (-3.2 - 5.0%). The single measures ICC for VO_2 between baseline and PLA trials was 0.90 (95% CI 0.79 - 0.96) indicating high reproducibility of the measurement method.

Maximal Repeated Sprints

Peak PO in Sprint 5 was higher in the BR trial than the PLA trial (PLA: 404 ± 24 W; BR: 420 ± 23 W, Figure 5), but there were no statistical differences in either peak PO, total work or fatigue index between trials ($p = .590$; $p = .667$; $p = .555$) or for Supplement $\times$ Sprint interaction ($p = .069$, $p = .177$, $p = .217$).

1 km Time Trial

TT completion time was not different between trials (Baseline: 276 ± 5 s, PLA: 277 ± 5 s, BR: 276 ± 5 s, $p = .539$, Figure 6). VO_2 was significantly lower in the BR trial during the TT compared with PLA ($p = .014$, 95% CI $0.5 - 2.8 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$). Post hoc analysis revealed that VO_2 was significantly reduced at the 400 m, 700 m, 800 m, and 900 m time points ($p < .05$). There was no differ-

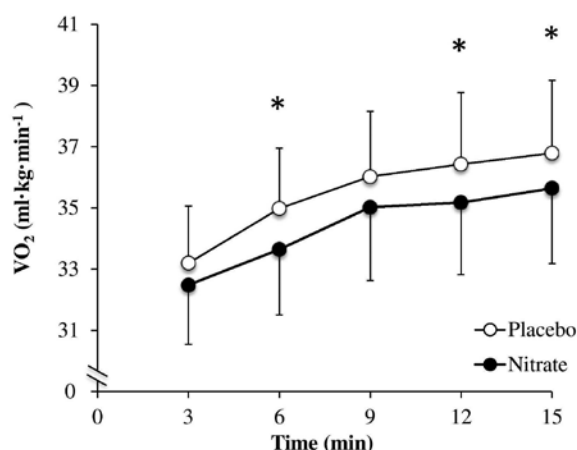


Figure 4 — Oxygen consumption during submaximal exercise following PLA (white circles) and BR (black circles) supplementation. Data are presented as the mean $\pm$ SEM (error bars); *Denotes significant difference between PLA and BR ($p = .010$).

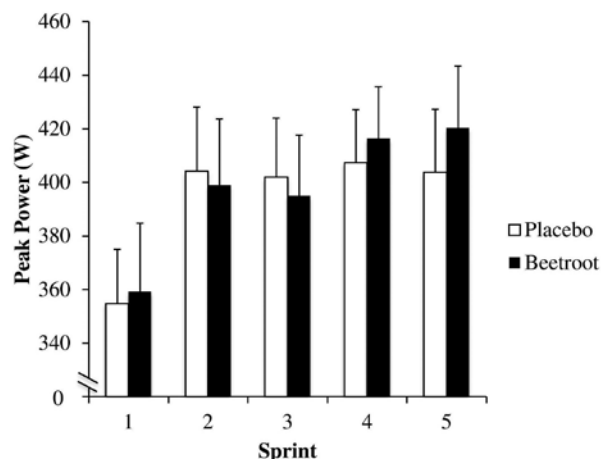


Figure 5 — Power output during repeated maximal sprints following PLA (white bars) and BR (black bars) supplementation. Data are presented as the mean $\pm$ SEM (error bars).

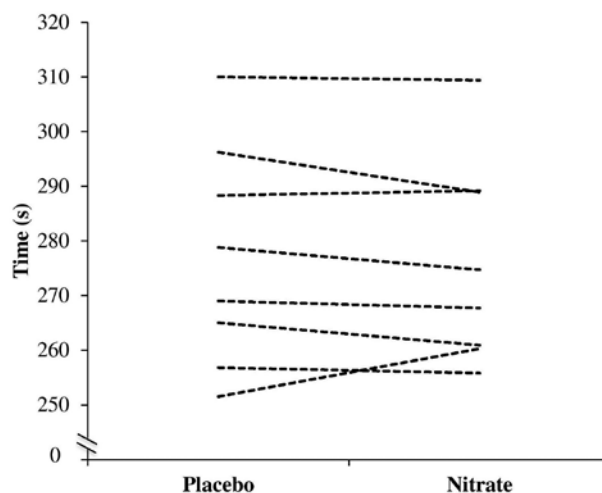


Figure 6 — times to complete 1 km TT following PLA (277 ± 5 s) and BR (276 ± 5 s) supplementation.

ence in either the peak VO_2 ($p = .128$), or the PO/VO_2 ratio ($p = .067$) during the TT between trials.

Discussion

To our knowledge this is the first study to investigate the effects of BR ingestion on the physiological responses, sprint and TT performance during kayaking. The principal finding of this study was that despite a reduction in the oxygen cost of submaximal steady-state exercise and high-intensity TT performance, acute supplementation of dietary nitrate through consumption of nitrate-rich BR did not enhance peak PO, work done or fatigue index during repeated maximal sprints or 1 km TT performance in

trained flatwater kayakers. This is in contrast to previous work that has demonstrated improvements in cycling, running, and rowing performance (Bond et al., 2012; Cermak et al., 2012a; Lansley et al., 2011a).

This study complements the findings of earlier investigations where dietary nitrate supplementation has been shown to reduce the oxygen cost of exercise (Bailey et al., 2009; Lansley et al., 2011a; Larsen et al., 2007; Vanhatalo et al., 2010). Novel data presented here demonstrates that a BR mediated reduction in VO_2 is also apparent during predominantly upper-body exercise. While the precise mechanism(s) underpinning the reduced metabolic cost of exercise remains elusive it is suggested to be caused by an NO derived improvement in muscle contractile efficiency (Bailey et al., 2010) or in the efficiency of mitochondrial respiration (Larsen et al., 2011). It must be acknowledged that the reduction in VO_2 observed following ingestion of BR was small (~3%) and within the 95% LOA for our measurement method. Although the measurement of VO_2 was shown to be highly reproducible during submaximal kayaking exercise (ICC = 0.90), the difference between BR and PL trials may simply be a consequence of the expected variability in VO_2 when testing athletes on different days, attributable to technical error and biological fluctuation (Armstrong and Costill 1985). Conversely, the reduction in VO_2 following BR supplementation is consistent with the vast majority of research published to date (Bailey et al., 2009, 2010; Lansley et al., 2011a; Larsen et al., 2007; Vanhatalo et al., 2010).

The extent of the reduction in VO_2 during kayaking (~3%) is similar to that of the study by Larsen et al. (2011) during cycling but approximately half of that demonstrated in other studies (Bailey et al., 2009; Vanhatalo et al., 2010). It could be argued that the smaller reduction in VO_2 was simply due to the smaller muscle mass used in upper-body exercise despite absolute blood flow being approximately the same for arm cranking and cycling at the same workload (Sawka, 1986). Consequently, an NO mediated increase in blood flow to the musculature of the upper-body may be less significant to that of the lower-body as there is a greater relative blood flow to the muscle during upper-body exercise. However, there is limited evidence to support this hypothesis and others who have used an exercise modality employing a small muscle mass (e.g., knee extensor exercise), still report larger reductions in VO_2 following nitrate supplementation (Bailey et al., 2010).

The smaller reduction in VO_2 during submaximal exercise may explain why, in contrast to previous research (Bond et al., 2012; Cermak et al., 2012a; Lansley et al., 2011b), dietary nitrate did not enhance 1 km TT performance in kayakers. The VO_2 data from the TT component of the experimental trials confirms that kayakers spend the majority of their race at or around their $\text{VO}_{2\text{peak}}$ during a 1 km effort (Bishop, 2000; Zamparo et al., 1999). Nevertheless, several other groups have also recently demonstrated that dietary nitrate supplementation did not enhance exercise performance, although these stud-

ies employed primarily endurance based performance tests with highly trained athletes (Bescós et al., 2012; Cermak et al., 2012b; Peacock et al., 2012; Wilkerson et al., 2012). There also appears to be little evidence to suggest that peak power, work done, or fatigue index in repeated upper-body supramaximal sprints is altered following nitrate supplementation.

There is, however, evidence to suggest that the smaller reduction in VO_2 and the lack of performance enhancement observed in the current study may be due to the method of nitrate delivery or the apparent variability in the individual response to nitrate supplementation. The fact that ingestion of BR did not reduce blood pressure, despite this being consistently reported on other occasions (Kapil et al., 2010; Vanhatalo et al., 2010; Webb et al., 2008), would seem to support this argument. While the majority of the initial BR studies employed a chronic loading period of dietary nitrate (0.5 L·day⁻¹ for 6 days [Bailey et al., 2009, 2010; Lansley et al., 2011a]), our method of nitrate delivery was to provide a single dose of BR 3 hr before the commencement of exercise which is similar to the design of Lansley et al. (2011b). These authors demonstrated that this protocol resulted in a substantial elevation of plasma nitrite and improvement of both 4 km and 16.1 km cycling TT performance. In the current study we found a single dose of nitrate-rich BR resulted in a 32% increase in plasma nitrite which is less than that shown by Lansley et al. (2011b; 138%) but similar to the increases reported by other studies (Bescós et al., 2011; Wilkerson et al., 2012) who employed the single BR dose protocol (16 and 25% respectively).

It is currently unknown as to why there is such a variation in the elevation of plasma nitrite following nitrate supplementation but it appears that chronic ingestion consistently results in a larger increase (95–140%; Bailey et al., 2009, 2010; Lansley et al., 2011a; Larsen et al., 2007; Larsen et al., 2010). The extent to which the elevation in plasma nitrite is associated with the subsequent reduction in VO_2 and enhancement of exercise performance is unclear at present as inconsistencies in the exercise intensity and modality between studies make comparisons difficult. Nevertheless, Wilkerson et al. (2012) reported a significant negative correlation between the difference in plasma nitrite after BR ingestion and the difference in 50 km cycling TT performance. As yet, there has been no further substantiation of this relationship although a responder/nonresponder issue with nitrate supplementation cannot be ruled out.

Evidence supporting the merits of a longer nitrate loading protocol can be found in a recent study by Hernández et al. (2012). The authors reported that seven days of dietary nitrate supplementation increases the force production of fast twitch muscle fibers during low stimulation frequency in mice, an effect that was not seen in slow twitch muscle fibers. The suggested mechanism for the increased force production was an alteration in muscle protein expression following a chronic dosage of dietary nitrate. This was evidenced by the increased expression of the calcium handling proteins calsequestrin

1 and dihydropyridine in fast twitch muscle fibers following dietary nitrate supplementation. Due to the reported greater reliance on fast twitch muscle fibers during upper-body exercise (Pendergast, 1989; Pimental et al., 1984) it would therefore be expected that ingestion of BR would have a positive effect on force production. Ferguson et al. (2012) also reported that five days of dietary nitrate supplementation elevated the delivery of O₂ to skeletal muscle in the fast twitch muscle fibers of rats. It should be noted that elite kayakers typically have a high percentage of slow twitch muscle fibers comparable to the lower body musculature of middle distance runners (Tesch & Karlsson, 1985) although in the absence of muscle biopsy samples it is impossible to determine the muscle fiber type distribution of the trained kayakers in this study. Although these effects are yet to be substantiated in humans, the majority of studies where an ergogenic effect of BR ingestion is reported (e.g., Bailey et al., 2009, 2010; Lansley et al., 2011a) use a 6-day loading period whereas this and other studies that used a single dose of BR do not (Bescós et al., 2012; Cermak et al., 2012b; Wilkerson et al., 2012). Intuitively therefore, although the acute dose of nitrate administered in the current study may have been adequate to increase NO bioavailability and reduce the metabolic cost of exercise, it may not be sufficient to alter muscle protein expression and therefore increase force generation. Recent data, however, suggests that the elevation in plasma nitrite is similar whether a single dose is ingested 2.5 hr before exercise or supplementation continued for 5 or even 15 days (Fulford et al., 2013).

Conceivably, the smaller rise in plasma nitrite may be mediated by two factors: 1) a single dose of dietary nitrate is not as effective as a chronic loading strategy or 2) a higher initial baseline level of circulating nitrite in some participants reduced the relative rise in plasma nitrite following ingestion of BR. Novel findings in the current data set would seem to support the latter suggestion, demonstrated by a strong negative correlation between baseline and % nitrite change following BR (Figure 3). Other studies where a high baseline level of nitrite is measured also typically result in a relatively small rise in plasma nitrite. For example, Wilkerson et al. (2012) reported a mean plasma nitrite at baseline of 379 nM followed by a 25% increase following nitrate supplementation. Conversely, Peacock et al. (2012) reported lower baseline plasma nitrite levels (143 nM) coupled with a larger increase (127%) following a single dose of potassium nitrate. Higher baseline concentrations of nitrite may be due in part to the participants habitually consuming a diet with high nitrate content or as a direct consequence of training status. For example, previous studies have demonstrated that the nitrate/nitrite pool is higher in endurance-trained athletes than in untrained matched controls (Jungersten et al., 1997; Schena et al., 2002). However, comparing nitrate and nitrite levels between different populations only allows us to speculate at the precise mechanism underpinning both the baseline level of plasma nitrite and the change in plasma nitrite following nitrate supplementation in trained individuals.

It is acknowledged that a limitation of the current study was that the consumption of nitrate rich foods in the days preceding each test was not limited. However, although the plasma nitrite levels in the baseline and PLA conditions were higher (513.40 ± 25.61 nM and 519.76 ± 25.82 nM respectively) than previously reported (typical range ~60–300 nM [e.g., Bailey et al., 2009; Bailey et al., 2010; Bescós et al., 2012; Cermak et al., 2012b; Lansley et al., 2011b; Larsen et al., 2007; Larsen et al., 2010; Peacock et al., 2012]), the increase of 32% following BR ingestion is still greater than that of other studies using a similar method of nitrate delivery (Bescós et al., 2011; Wilkerson et al., 2012). The higher levels of nitrite in our participants may be because they were trained as previously discussed (Jungersten et al., 1997; Schena et al., 2002) or due to differences in measurement techniques (Moshage et al., 1995). It is also worth noting that the majority of researchers have reported a decrease in plasma nitrite concentration following maximal intensity exercise (e.g., Larsen et al., 2010; Bescós et al., 2011). A potential limitation of this study is that plasma nitrite was not measured again before the start of the TT and therefore we cannot be certain that it was still substantially higher in the BR condition. Although VO₂ was lower during the TT component of the BR trial than in the PL trial, this does not confirm that plasma nitrite was elevated, partially due to the variability in PO during a TT. Indeed, a reduction in plasma nitrite toward baseline concentrations following the steady-state exercise and repeated sprints may partly explain why ingestion of BR did not enhance TT performance in the current study. Further studies which include additional measurements of plasma nitrite throughout the experimental trial or exclude the steady-state and repeated sprint sections are required to verify the present findings that BR does not enhance 1 km kayaking TT performance.

Conclusion

To our knowledge this is the first study that has investigated the effects of dietary nitrate on the oxygen cost of upper-body exercise and kayak performance. The primary outcome was that despite a reduction in VO₂ during submaximal and high-intensity kayaking exercise, a single dose of BR did not enhance repeated sprint or 1 km TT performance of trained flatwater kayakers. While the altered hemodynamic and metabolic response to upper-body exercise may account for these results there is little evidence to support this hypothesis. The lack of performance effect may be due in part to a smaller increase NO bioavailability following a single dose of BR in comparison with chronic nitrate loading protocols or a reduction in plasma nitrite before the TT mediated by the preceding repeated maximal effort sprints, although further studies are required to confirm this. Alternatively, the individual variability in response to nitrate ingestion, apparently mediated by the baseline plasma nitrite concentration, may also contribute to these findings.

Further research into the effects of nitrate on upper-body and repeated sprint exercise are required, along with a more detailed exploration of the issue of nonresponders to dietary nitrate supplementation.

References

- Armstrong, L.E., & Costill, D.L. (1985). Variability of respiration and metabolism: Responses to submaximal cycling and running. *Research Quarterly for Exercise and Sport*, 56, 93–96. doi:10.1080/02701367.1985.10608441
- Bailey, S.J., Winyard, P., Vanhatalo, A., Blackwell, J.R., DiMenna, F.J., Wilkerson, D.P., . . . Jones, A.M. (2009). Dietary nitrate supplementation reduces the O₂ cost of low intensity exercise and enhances tolerances to high-intensity exercise in humans. *Journal of Applied Physiology*, 107(4), 1144–1155. PubMed doi:10.1152/japophysiol.00722.2009
- Bailey, S.J., Fulford, J., Vanhatalo, A., Winyard, P.G., Blackwell, J.R., DiMenna, F.J., . . . Jones, A.M. (2010). Dietary nitrate supplementation enhances muscle contractile efficiency during knee-extensor exercise in humans. *Journal of Applied Physiology*, 109(1), 135–148. PubMed doi:10.1152/japophysiol.00046.2010
- Bescós, R., Rodríguez, F.A., Iglesias, X., Ferrer, M.D., Iborra, E., & Pons, A. (2011). Acute administration of inorganic nitrate reduces VO_{2peak} in endurance athletes. *Medicine and Science in Sports and Exercise*, 43(10), 1979–1986. PubMed doi:10.1249/MSS.0b013e318217d439
- Bescós, R., Ferrer-Roca, V., Galilea, P.A., Roig, A., Drobnic, F., Sureda, A., . . . Pons, A. (2012). Sodium nitrate supplementation does not enhance performance of endurance athletes. *Medicine and Science in Sports and Exercise*, 44(12), 2400–2409. PubMed doi:10.1249/MSS.0b013e3182687e5c
- Bishop, D. (2000). Physiological predictors of flatwater kayak performance in women. *European Journal of Applied Physiology*, 82(1–2), 91–97. PubMed doi:10.1007/s004210050656
- Bishop, D., Bonetti, D., & Spencer, M. (2003). The effect of intermittent, high-intensity warm-up on supramaximal kayak ergometer performance. *Journal of Sports Sciences*, 21(1), 13–20. PubMed doi:10.1080/0264041031000070912
- Bland, J.M., & Altman, D.G. (1986). Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet*, 1(8476), 307–310. PubMed doi:10.1016/S0140-6736(86)90837-8
- Bond, H., Morton, L., & Braakhuis, A.J. (2012). Dietary nitrate supplementation improves rowing performance in well-trained rowers. *International Journal of Sport Nutrition and Exercise Metabolism*, 22(4), 251–256. PubMed doi:10.1111/j.1748-1716.2007.01713.x
- Doel, J.J., Benjamin, N., Hector, M.P., Rogers, M., & Allaker, R.P. (2005). Evaluation of bacterial nitrate reduction in the human oral cavity. *European Journal of Oral Sciences*, 113(1), 14–19. PubMed doi:10.1111/j.1600-0722.2004.00184.x
- Eisenbrand, G., Spiegelhalter, B., & Preussmann, R. (1980). Nitrate and nitrite in saliva. *Oncology*, 37(4), 227–231. PubMed doi:10.1159/000225441
- Ferguson, S.K., Hirai, D.M., Copp, S.W., Holdsworth, C.T., Allen, J.D., Jones, A.M., . . . Poole, D.C. (2013). Impact of dietary nitrate supplementation via beetroot juice on exercising muscle vascular control in rats. *Journal of Physiology*, 591(Pt 2), 547–557. PubMed doi:10.1113/jphysiol.2012.243121
- Fulford, J., Winyard, P.G., Vanhatalo, A., Bailey, S.J., Blackwell, J.R., & Jones, A.M. (2013). Influence of dietary nitrate supplementation on human skeletal muscle metabolism and force production during maximum voluntary contractions. *Pflügers Archiv*, 10.1007/s00424-013-1220-5. PubMed doi:10.1007/s00424-013-1220-5
- Hernández, A., Schiffer, T.A., Ivarsson, N., Cheng, A.J., Bruton, J.D., Lundberg, J.O., . . . Westerblad, H. (2012). Dietary nitrate increases tetanic [Ca²⁺]_i and contractile force in mouse fast-twitch muscle. *Journal of Physiology*, 590(Pt 15), 3575–3583. PubMed doi:10.1113/jphysiol.2012.232777
- Jungersten, L., Ambring, A., Wall, B., & Wennmalm, A. (1997). Both physical fitness and acute exercise regulate nitric oxide formation in healthy humans. *Journal of Applied Physiology*, 82(3), 760–764. PubMed doi:10.1161/HYPERTENSIONA.110.153536
- Kapil, V., Milsom, A.B., Okorie, M., Maleki-Toyserkani, S., Akram, F., Rehman, F., . . . Ahluwalia, A. (2010). Inorganic nitrate supplementation lowers blood pressure in humans. *Hypertension*, 56(2), 274–281. PubMed doi:10.1161/HYPERTENSIONA.110.153536
- Kenjale, A.A., Ham, K.L., Stabler, T., Robbins, J.L., Johnson, J.L., Vanbruggen, M., . . . Allen, J.D. (2011). Dietary nitrate supplementation enhances exercise performance in peripheral arterial disease. *Journal of Applied Physiology*, 110(6), 1582–1591. PubMed doi:10.1152/japophysiol.00071.2011
- Lansley, K.E., Winyard, P.G., Fulford, J., Vanhatalo, A., Bailey, S.J., Blackwell, J.R., . . . Jones, A.M. (2011a). Dietary nitrate supplementation reduces the O₂ cost of walking and running: A placebo-controlled study. *Journal of Applied Physiology*, 110(3), 591–600. PubMed doi:10.1152/japophysiol.01070.2010
- Lansley, K.E., Winyard, P.G., Bailey, S.J., Vanhatalo, A., Wilkerson, D.P., Blackwell, J.R., . . . Jones, A.M. (2011b). Acute dietary nitrate supplementation improves cycling time trial performance. *Medicine and Science in Sports and Exercise*, 43(6), 1125–1131. PubMed doi:10.1249/MSS.0b013e31821597b4
- Larsen, F.J., Ekblom, B., Sahlin, K., Lundberg, J.O., & Weitzberg, E. (2007). Effects of dietary nitrate on oxygen cost during exercise. *Acta Physiologica (Oxford, England)*, 191(1), 59–66. PubMed doi:10.1111/j.1748-1716.2007.01713.x

- Larsen, F.J., Weitzberg, E., Lundberg, J.O., & Ekelund, B. (2010). Dietary nitrate reduces maximal oxygen consumption while maintaining work performance in maximal exercise. *Free Radical Biology & Medicine*, 48(2), 342–347. [PubMed doi:10.1016/j.freeradbiomed.2009.11.006](#)
- Larsen, F.J., Schiffer, T.A., Borniquel, S., Sahlin, K., Ekelund, B., Lundberg, J.O., & Weitzberg, E. (2011). Dietary inorganic nitrate improves mitochondrial efficiency in humans. *Cell Metabolism*, 13(2), 149–159. [PubMed doi:10.1016/j.cmet.2011.01.004](#)
- Lundberg, J.O., & Weitzberg, E. (2010). NO-synthase independent NO generation in mammals. *Biochemical and Biophysical Research Communications*, 396(1), 39–45. [PubMed doi:10.1016/j.bbrc.2010.02.136](#)
- Moshage, H., Kok, B., Huizenga, J.R., & Jansen, P.L. (1995). Nitrite and nitrate determinations in plasma: A critical evaluation. *Clinical Chemistry*, 41(6 Pt 1), 892–896. [PubMed](#)
- Paton, C.D., & Hopkins, W.G. (2006). Variation in performance of elite cyclists from race to race. *European Journal of Sport Science*, 6(1), 25–31. [doi:10.1080/17461390500422796](#)
- Peacock, O., Tjonna, A.E., James, P., Wisloff, U., Welde, B., Bohlke, N., . . . Sandbakk, O. (2012). Dietary nitrate does not enhance running performance in Elite cross-country skiers. *Medicine and Science in Sports and Exercise*, 44(11), 2213–2219. [PubMed doi:10.1249/MSS.0b013e3182640f48](#)
- Pendergast, D.R. (1989). Cardiovascular, respiratory, and metabolic responses to upper body exercise. *Medicine and Science in Sports and Exercise*, 21(5, Suppl) S121–S125. [PubMed](#)
- Pimental, N.A., Sawka, M.N., Billings, D.S., & Trad, L.A. (1984). Physiological responses to prolonged upper-body exercise. *Medicine and Science in Sports and Exercise*, 16(4), 360–365. [PubMed doi:10.1249/00005768-198408000-00007](#)
- Rogers, S.C., Khalatbari, A., Gapper, P.W., Frenneaux, M.P., & James, P.E. (2005). Detection of human red blood cell-bound nitric oxide. *Journal of Biological Chemistry*, 280(29), 26720–26728. [PubMed doi:10.1074/jbc.M501179200](#)
- Sawka, M.N. (1986). Physiology of upper body exercise. *Exercise and Sport Sciences Reviews*, 14, 175–211. [PubMed doi:10.1249/00003677-198600140-00009](#)
- Schena, F., Cuzzolin, L., Rossi, L., Pasetto, M., & Benoni, G. (2002). Plasma nitrite/nitrate and erythropoietin levels in cross-country skiers during altitude training. *Journal of Sports Medicine and Physical Fitness*, 42(2), 129–134. [PubMed](#)
- Stamler, J.S., & Meissner, G. (2001). Physiology of nitric oxide in skeletal muscle. *Physiological Reviews*, 81(1), 209–237. [PubMed](#)
- Tesch, P., Piehl, K., Wilson, G., & Karlsson, J. (1976). Physiological investigations of Swedish elite canoe competitors. *Medicine and Science in Sports*, 8(4), 214–218. [PubMed](#)
- Tesch, P.A., & Karlsson, J. (1985). Muscle fibre types and size in trained and untrained muscles of elite athletes. *Journal of Applied Physiology*, 59(6), 1716–1720. [PubMed](#)
- van Maanen, J.M., van Geel, A.A., & Kleinjans, J.C. (1996). Modulation of nitrate-nitrite conversion in the oral cavity. *Cancer Detection and Prevention*, 20(6), 590–596. [PubMed](#)
- Vanhatalo, A., Bailey, S.J., Blackwell, J.R., DiMenna, F.J., Pavey, T.G., Wilkerson, D.P., . . . Jones, A.M. (2010). Acute and chronic effects of dietary nitrate supplementation on blood pressure and the physiological responses to moderate intensity and incremental exercise. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, 299(4), R1121–R1131. [PubMed doi:10.1152/ajpregu.00206.2010](#)
- Vanhatalo, A., Fulford, J., Bailey, S.J., Blackwell, J.R., Winyard, P.G., & Jones, A.M. (2011). Dietary nitrate reduces muscle metabolic perturbation and improves exercise tolerance in hypoxia. *Journal of Physiology*, 589(Pt 22), 5517–5528. [PubMed](#)
- Webb, A.J., Patel, N., Loukogeorgakis, S., Okorie, M., Aboud, Z., Misra, S., . . . Ahluwalia, A. (2008). Acute blood pressure lowering, vasoprotective, and antiplatelet properties of dietary nitrate via bioconversion to nitrite. *Hypertension*, 51(3), 784–790. [PubMed doi:10.1161/HYPERTENSIONAHA.107.103523](#)
- Wilkerson, D.P., Hayward, G.M., Bailey, S.J., Vanhatalo, A., Blackwell, J.R., & Jones, A.M. (2012). Influence of acute dietary nitrate supplementation on 50 mile time trial performance in well-trained cyclists. *European Journal of Applied Physiology*, 112(12), 4127–4134. [PubMed doi:10.1007/s00421-012-2397-6](#)
- Xu, J., Xu, X., & Verstraete, W. (2001). Quantitative measurement of the nitrate reductase activity in the human oral cavity. *Food and Chemical Toxicology*, 39(4), 393–400. [PubMed doi:10.1016/S0278-6915\(00\)00150-2](#)
- Zamparo, P., Capelli, C., & Guerrini, G. (1999). Energetics of kayaking at submaximal and maximal speeds. *European Journal of Applied Physiology and Occupational Physiology*, 80(6), 542–548. [PubMed doi:10.1007/s004210050632](#)

Erratum

In the article by Muggeridge DJ, Howe C CF, Spendiff O, Pedlar C, James PE, Easton C, “The effects of a single dose of concentrated beetroot juice on performance in trained flatwater kayakers,” in the *International Journal of Sport Nutrition and Exercise Metabolism* 23(5), Figures 2 and 3 were incorrectly labeled due to an error in the production process. We apologize for the error. The online version of the article has now been corrected.