NRC Research Press

ARTICLE

Betalain-rich concentrate supplementation improves exercise performance and recovery in competitive triathletes

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Abstract: We aimed to determine the effects of a betalain-rich concentrate (BRC) of beetroots, containing no sugars or nitrates, on exercise performance and recovery. Twenty-two (9 men and 13 women) triathletes (age, 38 ± 11 years) completed 2 double-blind, crossover, randomized trials (BRC and placebo) starting 7 days apart. Each trial was preceded by 6 days of supplementation with 100 mg·day^{-1} of BRC or placebo. On the 7th day of supplementation, exercise trials commenced 120 min after ingestion of 50 mg BRC or placebo and consisted of 40 min of cycling (75 ± 5% maximal oxygen consumption) followed by a 10-km running time trial (TT). Subjects returned 24 h later to complete a 5-km running TT to assess recovery. Ten-kilometer TT duration (49.5 ± 8.9 vs. 50.8 ± 10.3 min, p = 0.03) was faster with the BRC treatment. Despite running faster, average heart rate and ratings of perceived exertion were not different between treatments. Five-kilometer TT duration (23.2 ± 4.4 vs 23.9 ± 4.7 min, p = 0.003), 24 h after the 10-km TT, was faster in 17 of the 22 subjects with the BRC treatment. Creatine kinase, a muscle damage marker, increased less (40.5 ± 22.5 vs. 49.7 ± 21.5 U·L^{-1}, p = 0.02) from baseline to after the 10-km TT and subjective fatigue increased less (−0.05 ± 6.1 vs. 3.23 ± 6.1, p = 0.05) from baseline to 24 h after the 10-km TT with BRC. In conclusion, BRC supplementation improved 10-km TT performance in competitive male and female triathletes. Improved 5-km TT performances 24 h after the 10-km TT and the attenuated increase of creatine kinase and fatigue suggest an increase in recovery while taking BRC.

Key words: beetroot, brief fatigue inventory, muscle damage markers, creatine kinase, fatigue, time trial.

Résumé : Cette étude se propose de déterminer les effets d'un concentré de bétalaïnes provenant de betteraves (« BRC ») et ne contenant pas de sucres et de nitrates sur la performance physique et la récupération. Vingt-deux triathloniens (13 femmes et 9 hommes) âgés de 38 ± 11 ans participent selon un devis croisé aléatoire à double insu à deux essais (BRC et placebo) à 7 jours d'intervalle. Chaque essai est précédé de 6 jours de supplémentation en BRC ou en placebo (100 mg·jour^{-1}). Le 7^{e} jour de la supplémentation, l'essai commence 120 min après la consommation de 50 mg de BCR ou du placebo et consiste en 40 min de pédalage (75 ± 5 % de la consommation maximale d'oxygène) suivi d'un contre-la-montre (« TT ») de 10 km à la course. Vingt-quatre heures plus tard, les sujets participent à un TT de 5 km pour l'évaluation de la récupération. Dans la condition BRC, la durée du TT de 10 km est plus brève (49,5 ± 8,9 versus 50,8 ± 10,3 min, p = 0,03). Même en présence d'une meilleure performance, la fréquence cardiaque moyenne et l'intensité perçue de l'effort ne diffèrent pas entre les deux conditions. Le temps de performance au TT de 5 km (23,2 ± 4,4 versus 23,9 ± 4,7 min, p = 0,003) 24 h après le TT de 10 km est meilleur dans la condition BRC chez 17 des 22 sujets. Dans la condition BRC, la créatine kinase, un marqueur des lésions musculaires, augmente moins (40,5 ± 22,5 vs 49,7 ± 21,5 U·L^{-1}, p = 0,02) du début à la fin du TT de 10 km et la fatigue subjective augmente moins (−0,05 ± 6,1 vs 3,23 ± 6,1, p = 0,05) du début jusqu'à 24 h après le TT de 10 km. En conclusion, la supplémentation en BRC suscite une amélioration de la performance au TT de 10 km chez des triathloniens de compétition, femmes et hommes. L'amélioration de la performance au TT de 5 km 24 h après le TT de 10 km et la moins grande augmentation de la créatine kinase et de la fatigue suggèrent une amélioration de la récupération par la consommation de BRC. [Traduit par la Rédaction]

Mots-clés : betterave, bilan abrégé de fatigue, marqueurs des lésions musculaires, créatine kinase, fatigue, contre-la-montre.

Introduction

Red beetroot is a popular natural supplement, due to its reported health and exercise performance benefits (Bond et al. 2012, 2014; Detopoulou et al. 2008; Hoon et al. 2014; Kelly et al. 2013*a*, 2013*b*). Beetroot's bioactivity has been thought to be due to high concentrations of nitrates and various phytochemicals, including betalains (Clifford et al. 2016). Both of these compounds are found in concentrated beetroot juice (BRJ) (5–9 mmol of nitrate or about 85 g of beets) (Clifford et al. 2016), which has been shown to decrease blood pressure at rest (Larsen et al. 2006), decrease oxygen consumption during submaximal exercise (Bailey et al. 2009; Lansley et al. 2011*b*), and improve running and cycling time-trial (TT) performance (Bailey et al. 2009; Cermak et al. 2012; Lansley et al. 2011*a*). Most of the effects of BRJ are attributed to its high nitrate content, which is thought to improve muscle blood flow and oxygenation (Bailey et al. 2009; Cermak et al. 2012; Lansley et al. 2011*a*, 2011*b*; Webb et al. 2008). However, research has shown that during rest, the betalains found in beetroots have antioxidant and anti-inflammatory properties (Albano et al. 2015; Georgiev et al. 2010; Gliszczynska-Swiglo et al. 2006; Kanner et al. 2001; Tesoriere et al. 2003, 2004*a*, 2005), which could reduce

Received 12 August 2016. Accepted 3 October 2016.

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exercise-related muscle cell damage and have beneficial exercise performance effects (Davis et al. 2007).

The strong antioxidant capabilities of betalains are thought to be due to their very stable structures and the ability of their phenol and cyclic amine rings to donate protons and electrons, allowing betalain to readily reduce reactive oxygen species (Kanner et al. 2001). Kanner et al. (2001) showed that betalains inhibited linolate peroxidation by cytochrome *c* to a greater extent than other flavonoids. The ability of betalains to neutralize superoxide radicals may lead to an increase in nitric oxide availability in the blood, and subsequently increased blood flow and oxygen delivery (Sakihama et al. 2012). Dietary antioxidants such as vitamin C and E, carotenoids, and flavonoids can counteract the cellular damage associated with exercise (Kanner et al. 2001; Tesoriere et al. 2004*a*). Davis et al. (2007) showed that curcumin, a constituent of the Indian spice turmeric, with antioxidant and anti-inflammatory properties similar to betalains, reduced muscle cell damage (measured by the muscle damage marker creatine kinase) and inflammation (measured by cytokines) induced by downhill running. Since betalains have been shown to exhibit antioxidant and anti-inflammatory properties at rest, they may be able to reduce exercise-induced inflammation, muscle damage, and perhaps improve exercise performance.

We know of only 1 study that examined the effects of betalains during exercise. Van Hoorebeke et al. (2016) showed improved 5-km TT performance in 10 of the 13 competitive male runners, and ratings of perceived exertion were lower with betalain-rich concentrates (BRC) of beetroot supplementation compared with the control. Lactate dehydrogenase, a marker of muscle damage, increased less from baseline to immediately after the 5-km TT with the BRC treatment, despite no differences in subjective measures of muscle soreness and fatigue. This study did not examine the effects of betalains on recovery.

Therefore, the purpose of this study was to examine the effects of a BRC of beetroot, which was depleted of sugar and nitrates, taken before exercise and after 6 days of "preloading" with 100 mg of BRC, on 10-km TT running performance, serum muscle damage markers, muscle soreness, and overall fatigue in competitive male and female triathletes. In addition, we investigated the effects of BRC on recovery by measuring 5-km running TT performance 24 h after the initial 10-km TT. It was hypothesized that the BRC supplementation would decrease serum markers of muscle damage and subjective muscle soreness and fatigue, and thus improve running TT performance and recovery compared with placebo.

Materials and methods

Subjects

We recruited 26 competitive triathletes (13 male and 13 female) from the University of California at Davis campus and local venues to participate in the study. Twenty subjects were needed based on a power analysis (power = 0.8, significance p = 0.05, mean difference (MD) = 0.6 min for performance time of BRC supplement versus placebo and SD of the MD = 0.9 min) from a pilot study with competitive runners. Three of the male subjects were excluded in the analysis because of noncompliance with the training requirements of the study and 1 male subject was unable to complete testing because of work conflicts. Therefore, only 22 of the 26 subjects' data (13 women and 9 men) were included in the analysis. Inclusion criteria required that participants exercise more than 5 h per week, have completed a triathlon (Olympic, half or full ironman) within the last year, be nonsmokers, and be healthy as determined by a health-history questionnaire. Written informed consent was obtained as approved by the Institutional Review Board of the University of California at Davis.

Experimental design

This was a crossover, double-blinded, placebo-controlled study. Subjects ingested blue and white pill capsules, identical in appearance, containing a BRC of beetroot (FutureCeuticals, Momence, Ill., USA: Serving size: 1 capsule (50 mg beetroot concentrate) 5 kcal, 0.1 mg protein, 1 mg carbohydrate, 0 mg fat, 0.3 mg fiber, and 12.5 mg betalains (Nemzer et al. 2011)) or placebo (Serving size: 1 capsule, Silica from Sigma–Aldrich, catalog no. 381276). Treatments were taken twice a day (~30 min before breakfast and dinner) beginning 6 days prior to each test day as 6 days of preloading with BRJ was shown to improving cycling TT performance (Cermak et al. 2012). The order of treatment was randomized using a computer program. Subjects were asked to come to the laboratory on 5 occasions over the course of 3 weeks. The first visit (day 0) consisted of a medical-clearance examination, a maximal exercise test, and a practice 10-km TT. Seven days and 14 days after the initial visit to the laboratory, subjects completed the exercise trials and then returned the next morning for a 5-km TT run on a treadmill and blood draw. Subjects were prohibited from supplementing their diets with vitamins or anti-inflammatories, including red and yellow beets, colored swiss chard, prickly pear, aspirin, and ibuprofen for the duration of the study.

Screening measures

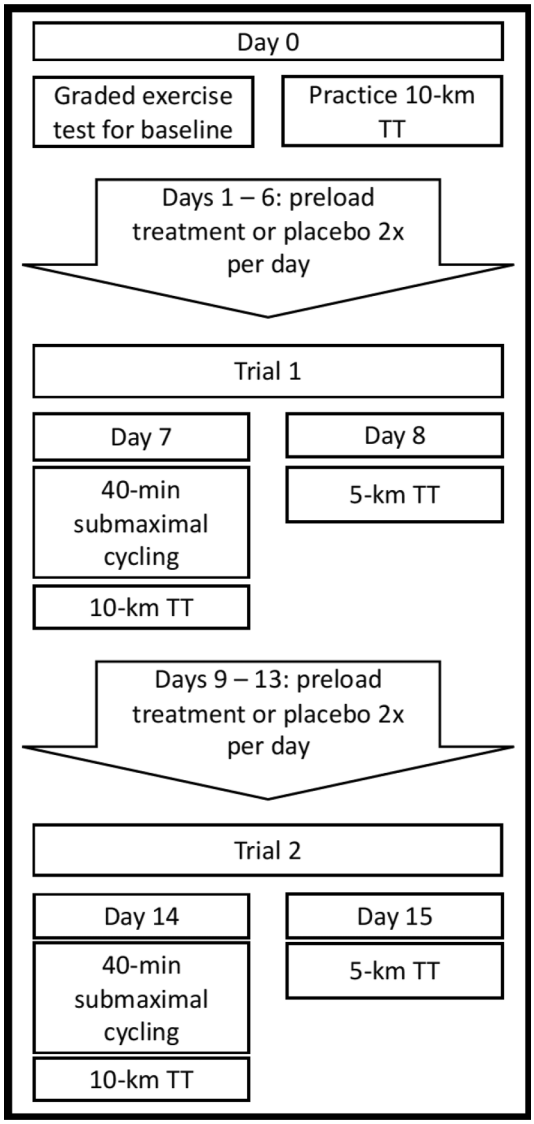
Upon arrival on day 0 we obtained measurements of height, body mass, and body composition (7-site skin fold; Jackson and Pollock 1978; Jackson et al. 1980). Subjects performed a standardized 10-min warm-up, on their own bicycles mounted on Computrainers (Computrainer Pro, Racemate Inc., Seattle, Wash., USA) followed by a graded exercise test to exhaustion to determine maximal oxygen consumption ($\dot{V}O_{2max}$). The initial workload of the test was 2 W·kg^{-1} to account for body mass and fitness level and was increased by 25–40 W every 3 min until volitional fatigue. A metabolic cart (TrueOne 2400, ParvoMedics, Sandy, Utah, USA) was used to make continuous metabolic measurements, while heart rate (HR) (model 5410, Polar, Woodbury, N.Y., USA) and ratings of perceived exertion (RPE) (10-point scale) (Noble et al. 1983) were measured every 3 min. The metabolic cart was calibrated prior to each trial at various flow rates (50–400 L·min^{-1}) and both room air and a standard gas mixture of 16% O_2 and 4% CO_2. Subjects then completed a practice 10-km TT on a treadmill (Stairmaster Clubtrack 2100 LE; Nautilus, Vancouver, Wash., USA). Subjects controlled their speed and had access to their distance, but were blinded to their actual speed and HR.

Experimental trials

The experimental protocol is depicted in Fig. 1. Prior to the first experimental trial, subjects recorded 7 days of training (training log; type, duration, intensity and km of training) and logged 3 days of their diet (day 5, 6, and 7) (MyFitnessPal Inc., San Francisco, Calif., USA). Subjects were given copies of their logs and asked to follow the diet and exercise exactly prior to the second trial. Subjects were asked to eat their normal pre-race breakfast, lunch, and dinner the day before testing and then eat a 190-kcal snack (Clif Bar, Emeryville, Calif., USA: 190 kcal, 68% carbohydrates (CHO), 16% fat, and 16% protein) around 2200 h the evening prior to testing to insure adequate glycogen stores for the experimental trials the next day. Subjects reported to the lab between 0830–1000 h in a fasted state (10 h; water only). Baseline muscle soreness and fatigue were recorded with 100-mm visual analogue scales (VAS) from "no pain" to "extreme pain" and from "not tired" to "utterly exhausted". Athletes also completed a brief fatigue inventory (BFI) consisting of 9 questions about the severity of fatigue and the effects of fatigue on physical ability and mood (Mendoza et al. 1999).

Baseline blood samples (9 mL), via a 22-gauge forearm vein catheter, blood pressure (BP), and HR were obtained. Following baseline measurements, 50 mg of BRC or placebo (inside an identical

Fig. 1. Experimental design. TT, time trial.



blue and white pill capsule) was given with 7 mL·kg^{-1} body mass of water. Subjects then rested for 120 min before commencing exercise to allow BRC to reach peak concentrations in the blood (Tesoriere et al. 2004*b*). At 30 min postsupplementation, subjects were provided a Smucker's Uncrustables, peanut butter and jelly sandwich (The T.M. Smucker Company, Orrville, Ohio, USA; 420 kcal: 56% CHO, 22% fat, and 22% protein) along with 3 mL·kg^{-1} body mass of water to prevent hypoglycemia and to simulate pretraining/competition behavior. At 110 min, subjects completed a 10-min standardized warm-up, voided their bladder, and had body mass measured (cycling attire, no shoes).

Submaximal exercise

Cycling exercise commenced at 120 min postsupplementation. Power (W) was estimated from the $\dot{V}O_{2max}$ test on day 0 to elicit 75% of $\dot{V}O_{2max}$ during the first 20 min. After 20 min, subjects consumed 3 mL·kg^{-1} of water and were connected to the metabolic cart. Workload was adjusted to match 75% of $\dot{V}O_{2max}$ and HR and RPE were measured at 30 and 40 min and then averaged for 1 submaximal exercise value. A blood sample, via a 22-gauge forearm vein catheter, and BP were taken during the last 2 min of exercise and 3 mL·kg^{-1} body mass of water was provided upon completion of the 40-min exercise bout. The same workloads adjustments were followed for the second experimental trial.

10-km TT

Following the submaximal exercise bout, subjects completed a 5-min active recovery at 100 W, changed into their running shoes, and then completed a 10-km running TT. Subjects controlled their speed and had access to their distance, but were blinded to their actual speed and HR. Speed, RPE, and HR were recorded every 1.67 km and then averaged to get one 10-km TT value. Blood, via a 22-gauge forearm vein catheter, and BP were collected immediately after the TT. Three milliliters per kilogram body mass of water was ingested while subjects completed 5 min of active recovery at 4.8 km·h^{-1}. Postexercise VAS scales and BFI questionnaires were completed. Before leaving, subjects ingested 50 mg of treatment with 3 mL·kg^{-1} body mass of water to maximize BRC's effects on recovery (Tesoriere et al. 2004*b*).

5-km TT

Subjects recorded their diet and exercise training for day 7. They repeated that diet for their second trial. Subjects were given a snack (Clif Bar; 190 kcal, 68% CHO, 16% fat; and 16% protein) to eat 2 h prior to returning to the lab the morning of day 8 to allow for some energy prior to the TT and to add consistency to the protocol. Subjects were asked to not consume anything other than the snack and water before arriving to the lab on day 8. At 24 h postexercise, VAS scales and BFI questionnaires were recorded followed by a 24-h postexercise blood draw via venipuncture. After the blood draw, subjects were given 3 mL·kg^{-1} body mass of water and then began a 10-min warm-up on the treadmill. Following the warm-up, subjects completed a 5-km TT where subjects controlled their speed and had access to their distance, but were blinded to their actual speed and HR. Speed, RPE, and HR were recorded every 1.67 km and then the average was recorded. Following the TT, a new set of pills, either BRC or placebo, were given to subjects to ingest for the subsequent week before arrival to the laboratory for trial 2. Trial 2 was repeated exactly as trial 1 but with a different treatment.

Blood analysis

Blood samples were collected in nonheparinized syringes. Blood lactate was determined with a portable analyzer (Lactate Plus, Nova Biomedical, Waltham, Mass., USA) and hematocrit was determined using microhematocrit tubes (Statspin, Norwood, Mass., USA)). Aliquots of blood were transferred to serum-separating tubes. The tubes then sat at room temperature for 30 min and were centrifuged at 3000 rpm for 10 min. One hundred microliters of serum was analyzed for serum glucose, creatine kinase (CK), and lactate dehydrogenase (LDH) levels using Metlyte 8 and Basic Metabolic reagent discs (Piccolo Xpress Chemistry Analyzer, Abaxis, Union City, Calif., USA).

Statistical analysis

The normality of distribution for each variable was tested using the Shapiro–Wilk test. All variables were normally distributed ($p > 0.05$). Paired t tests were used for baseline, submaximal exercise, and TT comparisons of HR, oxygen consumption ($\dot{V}O_2$), respiratory exchange ratio (RER), RPE, blood lactate, serum glucose, serum LDH, and CK as well as measures of whole-body muscle soreness and fatigue between treatments. Paired tests were also used to analyze the changes in the levels of fatigue, LDH and CK (StatView software, version 5.0.1, SAS Institute Inc., Cary, N.C., USA). Data are presented as means ± SD and significance was accepted at $p \leq 0.05$.

Results

Subject characteristics

Subjects were recreationally competitive triathletes, racing distances from Olympic to full ironman triathlons. There were no differences in weekly training volumes between treatments (BRC: 8.7 ± 3.7 h·week^{-1}; placebo: 8.6 ± 3.9 h·week^{-1}, p = 0.773). Total calories consumed (BRC: 2297 ± 576 kcal; placebo: 2262 ± 601 kcal; p = 0.426) and macronutrient proportions (BRC: 3.94 ± 1.38 g CHO·kg^{-1} body weight (BW), 1.35 ± 0.66 g fat·kg^{-1} BW, 1.62 ± 0.55 g protein·kg^{-1} BW; placebo: 3.94 ± 1.99 g CHO·kg^{-1} BW, 1.32 ± 0.66 g fat·kg^{-1} BW, 1.60 ± 0.57 g protein·kg^{-1} BW; p_{CHO} = 0.868, p_{fat} = 0.905, $p_{protein}$ = 0.493) were also not different in the 3 days preceding each treatment trial. Subject physical characteristics are presented in Table 1.

Baseline measures

Baseline values were not different between treatments with HR being 54 ± 9 and 54 ± 7 beats·min^{-1} (p = 0.832), systolic blood pressure (SBP) being 108.6 ± 9.4 and 109.4 ± 7.5 mm Hg (p = 0.848), blood lactate (0.95 ± 0.35 vs. 0.87 ± 0.34 mmol·L^{-1}; p = 0.418), serum glucose (4.83 ± 0.45 vs. 4.86 ± 0.39 mmol·L^{-1}; p = 0.677), and hematocrit (42.1% ± 4.0% vs. 41.9% ± 3.4%; p = 0.684) for the BRC and placebo treatments, respectively. Whole-body muscle soreness (9.7 ± 9.3 and 11.3 ± 10.7 mm out of 100 mm; p = 0.358) and whole-body fatigue (17.3 ± 13.3 and 17.1 ± 12.3 mm out of 100 mm; p = 0.961) for BRC and placebo, respectively, at baseline were not different between treatments. Baseline BFI (13.0 ± 10.8 and 12.7 ± 8.8 out of 90; p = 0.873) was also not different between treatments. Baseline LDH (144.5 ± 28.0 vs. 141.9 ± 20.6 U·L^{-1}; p = 0.576) and CK (207.5 ± 121.5 vs. 211.9 ± 121.7 U·L^{-1}; p = 0.863) for BRC and placebo, respectively, were also not significantly different between treatments at baseline.

Submaximal exercise

Data from the 40-min submaximal cycling bout is reported in Table 2. Average power and %$\dot{V}O_{2max}$ were similar between treatments. We found no significant differences between treatments in average HR, RER, RPE, and $\dot{V}O_2$ over the last 10 min of exercise or in SBP measured during the last 2 min of exercise. There were also no differences between treatments in serum glucose (4.69 ± 0.84 vs. 4.84 ± 0.65 mmol·L^{-1}; p = 0.459), blood lactate (2.68 ± 1.38 vs. 2.66 ± 1.37 mmol·L^{-1}; p = 0.904), hematocrit (43.9 ± 3.6 vs. 44.5% ± 3.5%; p = 0.193), LDH (160.9 ± 31.5 vs. 161.6 ± 27.4 U·L^{-1}; p = 0.878), or CK (221 ± 28.2 vs. 233.3 ± 133.7 U·L^{-1}; p = 0.663) for BRC and placebo, respectively.

10-km TT

Data from the 10-km running TT are presented in Table 3. BRC supplementation was associated with a 0.3 km·h^{-1} (2%) increase in average speed and a 78-s (3%) decrease in completion time versus placebo. Fifteen of the 22 subjects had improved TT times with BRC compared with placebo (Fig. 2). Despite running faster there was no significant differences in HR, SBP, or RPE. There were also no treatment differences in post 10-km TT values for blood lactate (3.6 ± 1.9 vs. 3.5 ± 1.9 mmol·L^{-1}; p = 0.482), serum glucose (5.91 ± 1.49 vs. 6.41 ± 1.55 mmol·L^{-1}; p = 0.207), and hematocrit (44.5 ± 3.7 vs. 44.4% ± 3.7%; p = 0.900) for the BRC and placebo treatments, respectively. Post 10-km TT LDH (179.5 ± 23.2 vs. 181.1 ± 27.1 U·L^{-1}; p = 0.582) and CK (248.0 ± 132.9 vs. 261.6 ± 139.11 U·L^{-1}; p = 0.601) for BRC and placebo, respectively, were not significantly different between treatments. The change in serum CK levels from baseline to immediately after the 10-km TT (40.5 ± 22.5 and 49.7 ± 21.5 U·L^{-1}, p = 0.021 for BRC and placebo, respectively) (Fig. 3) was lower with BRC treatment compared with placebo. The change in serum LDH levels from baseline to immediately after the 10-km TT (35.0 ± 17.4 and 39.2 ± 16.2 U·L^{-1}, p = 0.424 for BRC and placebo, respectively) was not different between treatments. Whole-body muscle soreness (25.2 ± 22.4 and 23.6 ± 17.1 mm out of 100 mm; p = 0.707) and whole-body fatigue (39.9 ± 26.7 and 38 ± 21.5 mm out of 100 mm; p = 0.653) for BRC and placebo, respectively, were also not different between treatments. BFI (18.6 ± 11.8 and 16.4 ± 9.2 out of 90; p = 0.196) for BRC and placebo, respectively, was also not different between treatments (Fig. 4). Subjects were well hydrated during the trials, as body weight changes from baseline to after the 10-km TT were minimal and not different between treatments (–1.2 ± 0.6 and –1.1 ± 0.5 kg for BRC and placebo, respectively; p = 0.706).

Table 1. Subject physical characteristics for 9 men and 13 women.

Variable	Mean ± SD
Age, y	38.0±11.3
Height, cm	170.2±8.0
Weight, kg	67.0±8.5
Body fat, %	15.8±6.2
Fat-free mass, kg	56.6±9.2
Fat mass, kg	10.5±4.1
$\dot{V}O_{2max}$, mL·kg^{-1}·min^{-1}	50.4±7.7
Power at maximum, W·kg^{-1}	4.1±0.7

Note: $\dot{V}O_{2max}$, maximal oxygen consumption.

Table 2. Physiological responses during the 40-min submaximal cycling bout.

Variable	BRC	Placebo	p
Average power, W	181.2±35.3	182.2±35.3	1.00
Heart rate, beats·min^{-1}	150.8±11.0	151.0±10.1	0.877
SBP, mm Hg	144.6±11.4	145.2±13.7	0.813
$\dot{V}O_2$, L·min^{-1}	2.51±0.49	2.52±0.54	0.914
%$\dot{V}O_{2max}$	74.7±4.8	74.4±4.3	0.689
RER	0.91±0.04	0.91±0.03	0.842
% Energy from carbohydrate	70.2±9.6	69.8±10.0	0.842
% Energy from fat	29.8±9.6	30.2±10.0	0.842
RPE	4.91±1.26	4.86±1.22	0.808

Note: Values are means ± SD for 9 men and 13 women. BRC, betalain rich concentrates; RER, respiratory exchange ratio; RPE, ratings of perceived exertion; SBP, systolic blood pressure; $\dot{V}O_2$, oxygen consumption; $\dot{V}O_{2max}$, maximal oxygen consumption.

Table 3. Physiological responses during the 10-km time trial (TT).

Variable	BRC	Placebo	p
Average speed, km·h^{-1}	12.4±2.3*	12.1±2.4	0.04
Time to complete the TT, min	49.5±8.9*	50.8±10.3	0.028
Average heart rate, beats·min^{-1}	161.7±11.3	161.9±12.4	0.902
RPE, 0–10 scale	5.8±1.2	5.7±1.1	0.869

Note: Values are means ± SD for 9 men and 13 women. BRC, betalain rich concentrates; RPE, ratings of perceived exertion.
*Significantly different from placebo.

Recovery values 24 h after the 10-km TT

The change in CK from baseline to 24 h after the 10-km TT (41.0 ± 84.6 and 44.8 ± 78.2 U·L^{-1}, p = 0.856, for BRC and placebo, respectively) (Fig. 3) was not different between treatments. The change in serum LDH levels from baseline to 24 h after the 10-km TT (–1.2 ± 17.0 and 1.4 ± 11.3 U·L^{-1}, p = 0.538 for BRC and placebo, respectively), were not different between treatments. Whole-body muscle soreness 24 h after the 10-km TT (9.9 ± 13.6 and 10.5 ± 13.7 mm out of 100 mm; p = 0.847 for BRC and placebo, respectively) was not different by treatment. Whole-body fatigue 24 h after the 10-km TT (4.1 ± 21.5 and 9.0 ± 14.4 mm out of 100 mm; p = 0.336 for BRC and placebo, respectively) was not different by treatment. The change in fatigue levels, using the BFI, from baseline to 24 h after the 10-km TT was significantly lower with BRC compared with placebo (–0.05 ± 6.1 and 3.23 ± 6.1 out of 90; p = 0.046 for BRC and control, respectively) (Fig. 4).

Fig. 2. Individual 10-km time-trial times with betalain-rich concentrate (BRC) and placebo for 22 subjects (9 men and 13 women).

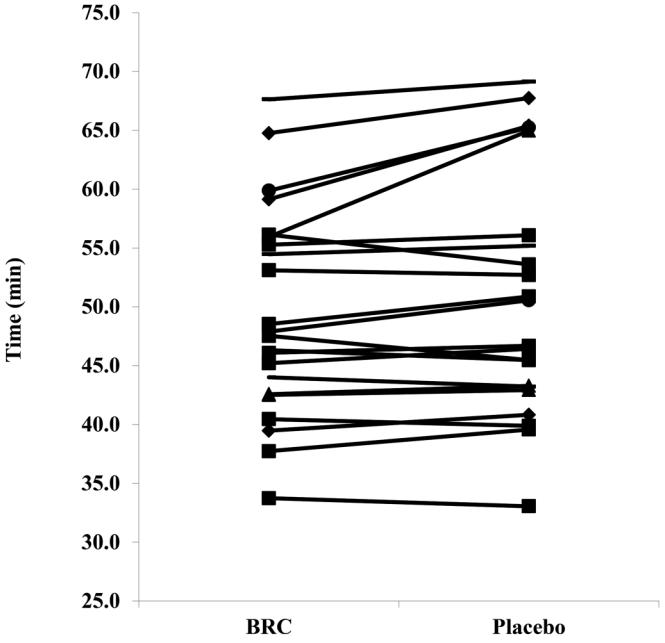


Fig. 3. The change in serum creatine kinase (CK) from baseline before supplementation to after the 10-km time trial (TT) and baseline before supplementation to 24 h after the 10-km TT. Data are means ± SD for 22 athletes (9 men and 13 women). *, Different from placebo; $p \leq 0.05$.

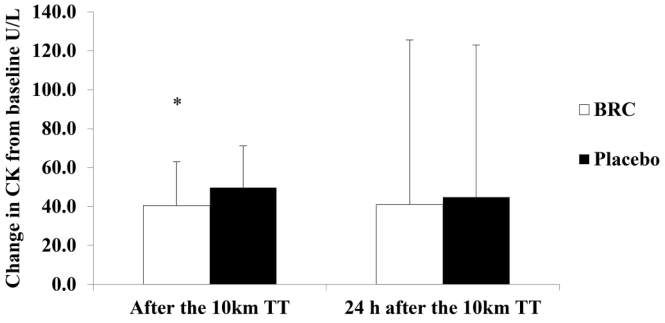


Fig. 4. The change in the brief fatigue inventory (BFI) score from baseline before supplementation to after the 10-km time trial (TT) and baseline before supplementation to 24 h after the 10-km TT. Data are means ± SD for 22 athletes (9 men and 13 women). *, Different from placebo; $p \leq 0.05$.

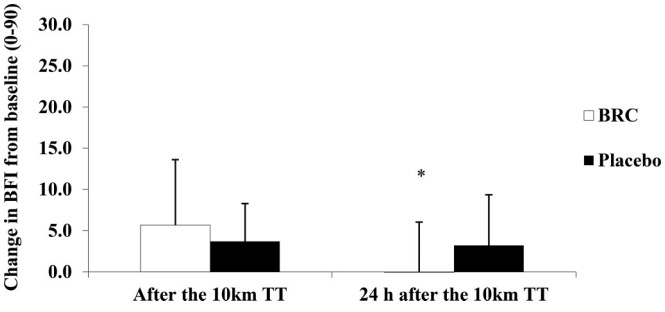


Table 4. Physiological responses during the 5-km time trial (TT).

Variable	BRC	Placebo	*p*
Average speed, km·h^{-1}	13.3±2.4*	12.9±2.4	0.001
Time to complete the TT, min	23.2±4.4*	23.9±4.7	0.003
Average heart rate, beats·min^{-1}	166.3±12.6*	162.7±14.5	0.0004
Average RPE, 0–10 scale	6.5±1.4	6.4±1.3	0.300

Note: Values are means ± SD for 9 men and 13 women. BRC, betalain rich concentrates; RPE, ratings of perceived exertion.
*Significantly different from placebo.

5-km TT

Data from the 5-km running TT conducted 24 h after the 10-km TT is presented in Table 4. BRC supplementation was associated with a 0.4-km·h^{-1} (3%) increase in speed and a 42-s (3%) decrease in time to completion versus placebo. Seventeen of the 22 subjects had improved TT times with BRC compared with placebo (Fig. 5). Despite running faster there was no significant differences in RPE, but HR was higher with BRC compared with control.

Discussion

The principal finding of this investigation was that supplementation with 50 mg of a BRC, the composition of which did not contain nitrates or sugars, 2 h prior to exercise and after 6 days of preloading, decreased 10-km running time in 15 of the 22 healthy, competitive male and female triathletes. In addition, serum CK, a marker of muscle damage, increased to a lesser extent from baseline to after the 10-km TT with BRC supplementation and fatigue also increased to a lesser extent (–0.05 ± 6.1 vs. 3.23 ± 6.1, $p = 0.05$) from baseline to 24 h after the 10-km TT with BRC. BRC supplementation also improved recovery as seen in faster 5-km TT times 24 h after the 10-km TT.

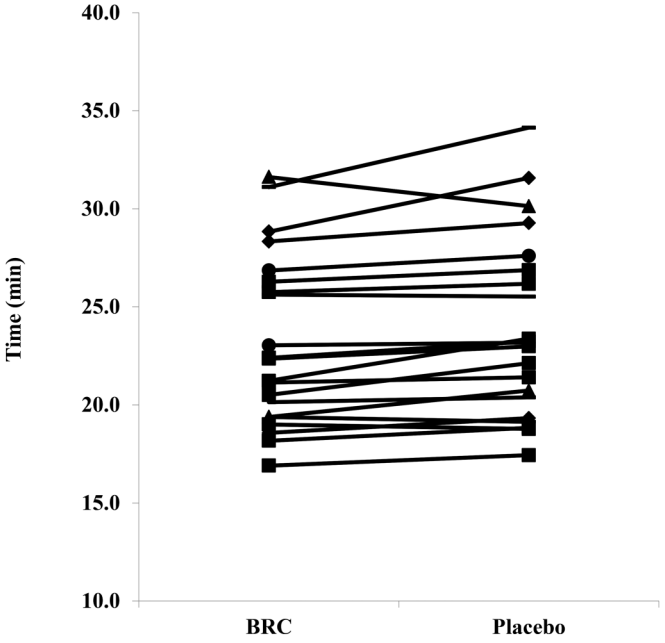
Submaximal exercise

Many studies have shown a decrease in $\dot{V}O_2$ and blood pressure during submaximal exercise when BRJ is supplemented to athletes (Bailey et al. 2009; Bond et al. 2014; Larsen et al. 2006; Wylie et al. 2013). The mechanism for decreased $\dot{V}O_2$ and lower blood pressure was thought to be due to the high levels of nitrates, which increase nitric oxide levels, vasodilation, and improved oxygen delivery (Bailey et al. 2009; Bond et al. 2014; Webb et al. 2008). Therefore, and as expected, because of the lack of nitrates, we saw no difference in $\dot{V}O_2$ or blood pressure when BRC alone was supplemented to triathletes. Van Hoorebeke et al. (2016) also did not find any differences in $\dot{V}O_2$ during submaximal exercise at 77% of $\dot{V}O_{2max}$ with BRC versus control using competitive runners. Similar to the Van Hoorebeke et al. (2016) study, we saw no effect of BRC on RER, supporting the idea that the changes in these variables when supplementing with beetroot juice are likely due to nitrates during submaximal exercise. However, that study found lower HRs during submaximal running at the same intensity, while we did not find a difference in submaximal HR during cycling. Mode of exercise or the higher fitness level of the triathletes in our study may explain these differences.

10-km TT

There have been several studies showing improved endurance exercise performance with BRJ supplementation (Cermak et al. 2012; Lansley et al. 2011*a*; Murphy et al. 2012). Lansley et al. (2011*a*) used a randomized, double-blinded, crossover study design and found that 1 dose of BRJ (6.2 mmol nitrates) given 2.75 h prior to exercise was associated with a 2.8% decrease in both 4-km and 16.1-km TT cycling time in competitive cyclists compared with a nitrate-depleted BRJ that served as a control. Cermak et al. (2012) found 6 days of supplementation with BRJ (8 mmol nitrates) in competitive cyclists improved 10-km cycling TT performance compared with a nitrate-depleted BRJ. Murphy et al. (2012) found that consumption of nitrate-rich whole beetroot improved 5-km TT

Fig. 5. Individual 5-km time-trial times with betalain-rich concentrate (BRC) and placebo for 22 subjects (9 men and 13 women).



running performance in recreationally fit men and women. The improved performance in these studies was thought to be related to the nitrates in the BRJ.

We know of only 1 study specifically examining the effects of betalains on exercise performance. Van Hoorebeke et al. (2016) found faster 5-km TT times with BRC versus control in 10 of the 13 competitive runners enrolled in the study. The 10-km TT times in our study were also significantly reduced during BRC supplementation compared with placebo. These results indicate that BRC supplementation, without typical beet sugars or nitrates, also has a beneficial effect on endurance athletes' TT running performance. Since both the nitrate-containing BRJ and the nitrate-depleted BRJ contained betalains, it is possible that the performance benefits of the nitrate-containing BRJ may have been even greater if a placebo had been used as a control instead.

The anti-oxidative and anti-inflammatory qualities of BRC (Albano et al. 2015; Georgiev et al. 2010; Gliszczynska-Swiglo et al. 2006; Kanner et al. 2001; Tesoriere 2003, 2004*a*, 2005) could potentially be reducing damage in the working muscle, as supported by an attenuation in CK increase from baseline to the conclusion of the 10-km TT in our study. This decrease in damage could lead to greater force-generating capability and lower levels of fatigue, although this is speculation and would need further measures of muscle damage and fatigue, such as muscle biopsies and peak force measurement, to make a stronger argument.

5-km TT, 24-h after 10-km TT

Five-kilometer TT times at 24 h after the 10-km TT were significantly faster following BRC supplementation compared with placebo. As a marker of recovery, the 5-km TT shows that BRC may be responsible for increased levels of recovery after a hard bout of exercise, and the HR differences show that BRC allows for higher intensity exercise even though perceived exertion did not change. BRC supplementation may have led to the blocking of superoxide radicals created during the submaximal exercise bout and 10-km TT, leading to greater amounts of available nitric oxide to vasodilate the working muscle and increase blood flow after exercise (Sakihama et al. 2012). Attenuation of the increase in fatigue from baseline to 24 h after the 10-km TT as measured by the BFI could also be due to decreased cell and muscle damage. It is possible that the increase in 5-km TT performance could have been due to the persistent ergogenic effects of BRC although this is unlikely as betalains have been shown to peak in the blood 3 h after ingestion and to disappear from the blood at 12 h post-ingestion (Tesoriere et al. 2004*b*).

Limitations and future directions

One limitation in this study was quantifying subjects' damage and recovery data. Although many subjects claimed to feel more energetic and more rested while on BRC, these opinions were not reflected in the VAS soreness and fatigue questionnaires. To have a better idea of the mechanism for this supplement, more quantifiable ways of assessing muscle damage, such as muscle biopsies, must be incorporated. Circulating blood markers of oxidative stress and nitric oxide metabolites should also be assesses in future studies to attain more information on the possible mechanisms for the improved running performance with betalain supplementation. Another limitation of this study may have resulted from too low of a betalain dose (~13 mg) compared with concentrations found in 100 g of whole beetroot (~93 mg) (Clifford et al. 2016). We may have found larger improvements in performance and greater reductions in muscle damage had we used a larger dose. However, we must again acknowledge that the profile of betalains provided by this BRC is different from profile of betalains found in beet root alone or in BRJ and, therefore, may be more bioavailable and/or may exert greater physiological effects. We did not verify the betalain content of the product or that it was free of nitrates. Future studies should measure the actual contents of the product as well as the blood concentration of betalains to verify betalains effect on exercise performance.

Furthermore, the type of exercise chosen (75% submaximal cycling exercise and 10-km running TT) may not have been long enough or intense enough to cause significant muscle damage in these well-conditioned athletes. We most likely would have seen a more robust response if we used eccentric exercise of higher intensity and duration, but that is not what triathletes typically do and is less applicable to a real-life situation. Exercise that involves more eccentric muscle contractions should be evaluated in future studies. Measurements of serum nitric oxide may have shed light on potential mechanisms for our findings, while measurements of serum BRC would have improved the validity. Thus, further studies should aim to incorporate higher concentrations of BRC and eccentric exercises to expand the conclusions of this study.

Conclusion

Low-dose BRC supplementation, preloaded for 6 days and 2 h prior to exercise, significantly decreased 10-km running TT the day of supplementation and decreased 5-km running TT the following day. BRC supplementation also reduced markers of muscle damage and attenuated increases in fatigue, but subjective measures of muscle soreness were unchanged. Therefore, given the improved 10-km TT performance and increased recovery we conclude that BRC supplementation can improve exercise performance and recovery in healthy, competitive triathletes.

Conflict of interest statement

Financial support for this study was provided by VDF FutureCeuticals, Inc. The study design, implementation, data interpretation and manuscript preparation were done without input from the sponsor.

Acknowledgements

We thank Kimya Baradaran, Kimberly Chong, Jose Hernandez, Danna Juarez-Rios, Brian Trinh, Eric Jones, and Emily Erlenbach for their assistance in data collection. We would also like to thank Dr. Liz Applegate and Dr. Keith Baar for their help editing the final manuscript. Most importantly, we appreciate the hard work and dedication of the subjects.

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