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ORIGINAL ARTICLE

Blackcurrant extract does not affect the speed–duration relationship during high-intensity running

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Abstract
Anthocyanin-rich blackcurrant extract (BC) has been shown to ergogenically aid high-intensity exercise. Capacity for such exercise is evaluated by the hyperbolic speed–tolerable duration ($S–D_{tol}$) relationship. Therefore, in double-blinded and cross-over randomised controlled trials, 15 males underwent treadmill running incremental exercise testing and were assessed for $S–D_{tol}$, quantified by critical speed (CS) and *D*' (distance), and assessments of time to exhaustion performance to empirically test the limits of the $S–D_{tol}$ relationship, after daily supplementation of 300 mg/d BC (105 mg/d anthocyanin) or placebo. Supplementation with BC did not change CS (placebo 12.1 ± 1.0 km/h vs BC 11.9 ± 1.0 km/h, $p > .05$) or *D*' (placebo 918.6 ± 223.2 m vs BC 965.2 ± 231.2 m, $p > .05$), although further analysis indicated *D*' increased in 60% of subject ($p = .08$), indicating a trend toward cohorts potentially benefiting from BC supplementation. BC supplementation did not change time to exhaustion at or above CS, maximal oxygen uptake (VO_{2max}), lactate threshold (LT), submaximal running economy (C_R), or substrate utilisation during exercise (all $p > .05$). In conclusion, we could not detect any beneficial effect of BC supplementation during high-intensity running exercise, including the determining factors $S–D_{tol}$ relationship, VO_{2max}, LT or C_R. Hence, no ergogenic effect was observed.

Keywords: *Anthocyanin, blackcurrant extract, high-intensity exercise, critical speed, speed–tolerable duration*

Highlights
- Blackcurrant extract containing anthocyanins is used as a nutritional supplement to enhance exercise performance, and perhaps especially intense exercise.
- Intense exercise lends itself to be examined by the speed-duration relationship, but which has not previously been used to evaluate blackcurrant extract.
- This study used the speed-duration relationship to evaluate the effect of blackcurrant extract, but could not find any effect of the supplement on exercise.

Introduction

Anthocyanins, of the flavonoid group of polyphenols, occur naturally in blackcurrant (Ribes Nigrum, BC) and its concentrated extract. This BC extract has emerged as a commercially available and legal sports supplement with purported ergogenic effects. A full and complete understanding of the phenomenon has not yet been fully established, but the observed effects relate to both health and exercise performance (Cook & Willems, 2019; Ehala, Vaher, & Kaljurand, 2005; Hurst et al., 2019).

Several mechanisms of action have been considered. The strongest evidence suggest that anthocyanins (1) provide anti-oxidant actions that reduce oxidative stress (Ehala et al., 2005; Hurst et al., 2019; Wallace, 2011); (2) protect the endothelium, promote nitric oxide bioavailability and facilitate endothelial-dependent vasorelaxation (Speciale, Cimino, Saija, Canali, & Virgili, 2014; Suhr, Gehlert, Grau, & Bloch, 2013; Yamagata, 2019); (3) increase the protective immune responsiveness to potential pathogens (Hutchison, Flieller, Dillon, & Leverett, 2016; Lyall et al., 2009); and (4) increase tissue fat oxidation (Cook, Myers,

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Blacker, & Willems, 2015; Strauss, Willems, & Shepherd, 2018). Thus, these actions have several health implications as they first and foremost protect against conditions exaggerated by oxidative stress and endothelial dysfunction such as cardiovascular disease, diabetes, certain cancers and degenerative diseases (Hurst et al., 2019; Matsumoto et al., 2005; Speciale et al., 2014; Suhr et al., 2013; Wallace, 2011; Yamagata, 2019). Secondly, they reduce inflammations (Lyall et al., 2009), and thirdly, they help manage metabolic problems and overweight (Cook et al., 2015; Strauss et al., 2018). Further studies have indicated that BC and anthocyanin supplementation also may confer broad-spectrum positive acid/base effects (Kessler, Jansen, & Hesse, 2002), anti-bacterial and anti-viral effects (Knox, Suzutani, Yosida, & Azuma, 2003; Puupponen-Pimia et al., 2001), certain regenerative effects (Matsumoto, Nakamura, Tachibanaki, Kawamura, & Hirayama, 2003), while also reducing exercise-induced cell and tissue damage (Hutchison et al., 2016; Lima, Barreto, Bassan, Greco, & Denadai, 2019; Lyall et al., 2009), tumour development and proliferation (Olsson, Gustavsson, Andersson, Nilsson, & Duan, 2004) as well as pain, fatigue and morbidity (Edwards, Blackburn, Christie, Townsend, & David, 2000; Leventhal, Boyce, & Zurier, 1994). However, it is not always clear whether the observed action was caused by anthocyanins and their conjugates, other anthocyanin-derived metabolites, or other compounds or derivatives from the anthocyanin source (Cook & Willems, 2019; Ehala et al., 2005).

The present study was however based on the notion that supplementation of anthocyanin-rich BC extract may also improve exercise performance. This is because, following from the abovementioned mechanisms, anthocyanins in effect may also increase skeletal muscle blood flow and microperfusion, increase blood lactate clearance, and improve skeletal muscle metabolic, contractile and recovery status (Cook & Willems, 2019; Cook, Myers, Gault, Edwards, & Willems, 2017a; Hurst et al., 2019; Hutchison et al., 2016; Lima et al., 2019; Perkins, Vine, Blacker, & Willems, 2015; Strauss et al., 2018; Willems, Myers, Gault, & Cook, 2014; Willems, Myers, Gault, & Cook, 2015), and thereby provide substrate to enhance exercise capacity and recovery. As such, anthocyanin-rich BC supplementation has been evidenced to increase exercise performance, at least under certain conditions. In a series of studies, Willems and colleagues showed that supplementation of mainly 300 mg/day New Zealand Blackcurrant Extract (105 mg/day anthocyanins) improved (1) 16.1 km (~28–29 min) cycling time-trial performance by 2.4% (44secs; Cook et al., 2015) (2) high-intensity intermittent cycling time trial performance, measured as total time to completion, by ~1% (3–4 s in each 4 km trial; Murphy, Cook, & Willems, 2017) (3) high-intensity intermittent sprint running performance, measured as individual and accumulated sprint distances, by 10–11% (Perkins et al., 2015); and (4) high-intensity intermittent shuttle running sprint performance, measured as reduced decrement in sprint time from first to last sprint by 40–50% (Godwin, Cook, & Willems, 2017; Willems, Cousins, Williams, & Blacker, 2016), compared to placebo.

In contrast to maximal exercise, submaximal and prolonged exercise (Willems et al., 2015; Willems, Parktin, Widjaja, & Ajjimaporn, 2018) or similar 16.1 km time-trials to that above, but under conditions of simulated altitude of ~2500 m with consequently reduced exercise intensity (Willems, Sahin, Berendsen, & Cook, 2019), BC did not facilitate any benefit. Taken together, it appears that benefits of anthocyanin-rich BC supplementation may be restricted to exercise performed at high and very high intensities, in which exercise is restricted to ~30mins or shorter durations before task failure occurs.

The above therefore immediately suggests that supplementation with anthocyanin-rich BC extract may facilitate high-intensity exercise performance via the critical power (CP; cycling) or speed (CS; running) concept. This concept relates to exercise with fixed work-rates and assumes that once a threshold (CP or CS) has been exceeded, tolerable duration may be predicted by the specific work-rate being performed. This is because the power– or speed–tolerable duration (S–D_{tol}) relationship is fixed to a hyperbolic function that may be accurately calculated, and it therefore also accurately describes the highest sustainable exercise work-rate in the severe intensity domain that may be tolerated for up to a maximum of ~30mins before task failure, with the quantity above the threshold measured by achievable work (*W*'; cycling) or distance (*D*'; running) (Burnley & Jones, 2018; Ferguson, Wilson, Birch, & Kemi, 2013; Poole, Burnley, Vanhatalo, Rossiter, & Jones, 2016). Only this work or distance is achievable, because the muscle glycogen and phosphocreatine levels that allow for attaining *W*' or *D*' fall to limiting values or work is compromised by accumulation of K^+, H^+, P_i, adenosine diphosphate, and reactive oxygen species that inexorably disturb intramuscular metabolic and contractile homeostasis (Burnley & Jones, 2018; Poole et al., 2016). For fixed-speed running, this therefore predicts performance as pacing above the CS will unavoidably cease in a predictable manner and is as such of value for evaluating exercise for exhaustive running up to ~30mins.

The aim of this study was therefore to examine the effect of daily anthocyanin-rich BC extract on S–D_{tol} during running. The hypothesis was that BC supplementation would right-shift the S–D_{tol} in the

severe exercise domain and thereby increase CS and *D*′; i.e. allow for longer-duration exercise at the same speed and/or allow for higher sustainable speed for the same duration of severe-intensity exercise.

Materials and methods

Subjects

Fifteen recreationally active males not engaged in structured training programmes volunteered, with characteristics in Table I. All were health-screened and familiarised to the experiments and signed informed consent forms prior to participation. Exclusion criteria included regular smoking, medication, drugs and pre-existing ergogenic aids, pre-existing medical conditions contraindicative to exercise testing, as well as alcohol and exhaustive exercise within 48 h. Breakfast was consumed 2 h before each test, with food and fluids except water avoided after this. The Institutional Review Board approved the study and it conformed to the Declaration of Helsinki.

Experimental design

Subjects attended the laboratory in 2 7-test blocks for intervention and placebo control respectively, with each test approximately at the same time of day (±2 h) and separated by >48 h and with blocks separated by a 14-day wash-out period, following a double-blind, randomised, placebo-controlled and cross-over design. Subjects warmed-up with a 10-min, 6 km/h, 1% gradient treadmill brisk walk/jog (PPS Med. Woodway, Weil am Rhein, Germany). Then, exercise testing commenced with, in order, an incremental exercise test (test 1), assessment of CS and S–D_{tol} (tests 2–5), and time to exhaustion performance trials (tests 6–7). Subjects were administered 300 mg/d BC extract (105 mg/d anthocyanin; CurraNZ New Zealand Blackcurrant Extract, Health Currancy, Surrey, UK) or placebo (300 mg/d microcrystalline cellulose M102), with $n = 8/15$ starting on BC extract and $n = 7/15$ on placebo, taken daily with breakfast from day −1 and throughout (thus 1-day pre-loading). This regime was adopted due to absorption and plasma anthocyanin peaking 2 h post-ingestion and clearance within 6–12 h (Hurst et al., 2019), as well as return to normal status within the wash-out period (Alvarez-Suarez et al., 2014). Nutritionally, 300 mg/d BC extract also provided 1 kcal/day, while placebo provided 0kcal/d. Subjects were instructed to maintain their normal diet including breakfast and schedule as consistent as possible and received no information of their results until after completing all trials.

Resting measurements

Subjects supine-rested for 10 mins before measurements of stature, blood pressure, and blood lactate (La^-) concentration ([La^-]). Body, fat, and lean masses were measured by air-displacement plethysmography (BodPod, Cosmed).

Incremental exercise test

A step-wise ramped incremental exercise test with speed increasing 0.5 km/step until volitional exhaustion measured submaximal running economy (C_R), La^- threshold (LT), maximal oxygen uptake (VO_{2max}), and maximal heart rate (HR_{max}). Fingerprick capillary blood [La^-] (Analox GM7 Lactate Analyzer, Analox, Hammersmith, UK), oxygen uptake (VO_2) by 1-min Douglas bag-collected expired gas samples (Servomex 4100 Gas Analyser, Servomex, Sussex, UK), and heart rate (HR; Polar Heart Rate S610i, Polar, Kempele, Finland) were measured at end of each step, with step-durations 5mins/step until LT was

Table I. Subject characteristics ($n = 15$)

Age (years)	24.4 ± 3.6
Height (m)	1.76 ± 0.06
Body mass (kg)	74.3 ± 6.7
Body fat (%)	10.8 ± 3.5
Fat mass (kg)	8.3 ± 2.4
Lean mass (kg)	66.0 ± 6.8
Body mass index ($kg \cdot m^{-2}$)	24.0 ± 0.8
VO_{2max} ($mL \cdot kg^{-1} \cdot min^{-1}$)	53.1 ± 3.4
HR_{max} (bpm)	194 ± 6
Systolic blood pressure (mmHg)	119.1 ± 6.1
Diastolic blood pressure (mmHg)	82.7 ± 7.0

Notes: Data are mean ± standard deviation (SD). VO_{2max}: maximal oxygen uptake; HR_{max}: maximal heart rate. Values are from placebo, and did not change with blackcurrant extract (BC) supplementation ($p > .05$).

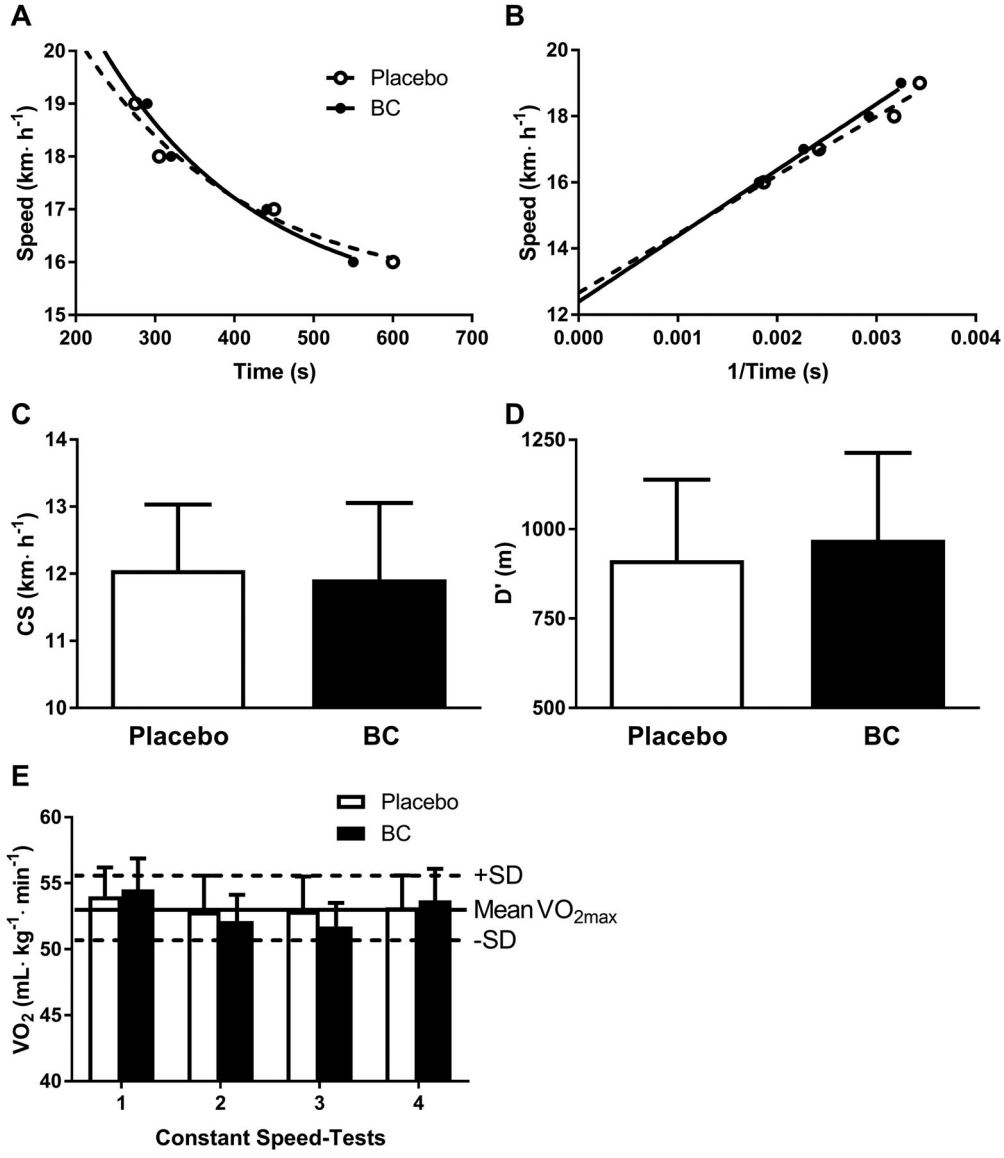

Figure 1. Characterisation of the Speed–tolerable duration ($S–D_{tol}$) relationship during running after placebo or supplementation with anthocyanin-rich blackcurrant extract (BC): (A) example traces of the hyperbolic nature of the $S–D_{tol}$ relationship; (B) example traces of quantification of critical speed (CS; intercept) and *D*' (slope) as parameters for $S–D_{tol}$; (C) critical speed (CS); (D) *D*'; E: mean and ±1 standard deviation (SD) of maximal oxygen uptake (VO_{2max}) measured during incremental exercise test (full and dotted horizontal lines), and oxygen uptake (VO_2) measured close to exercise intolerance during each constant speed-test (bars), VO_{2max} was attained in each constant speed-test. No differences occurred between placebo and BC or between incremental exercise test and constant speed-tests ($p > .05$).

identified and 1 min/step thereafter. LT was determined by the deflection point of La^- increase, while C_R was assessed at 8 km/h, LT, and 10 km/h. Energy expenditure and substrate utilisation rates were estimated at LT by indirect calorimetry (Frayn & Macdonald, 1997), and VO_{2max} was considered achieved when at least 3 of the following 4 criteria were met: VO_2 plateau, respiratory exchange ratio >1.15, post-exercise $[La^-]$ >8 mM, or HR within 10bpm of age-predicted maximum (Ferguson et al., 2013).

Speed–tolerable duration

The $S–D_{tol}$ relationship was estimated from at least 4 randomised and individualised 1%-inclined treadmill constant speed-tests, each separated by >48 h and designed to induce volitional exhaustion and exercise intolerance within ~3–20 min (Figure 1(A)). CS (intercept) and *D*' (slope) as parameters for $S–D_{tol}$ were calculated by least squares linear regression of the linear $S–D_{tol}^{-1}$ relationship (Figure 1(B)):

(Ferguson et al., 2013)

$$S = (D'/t_{tol}) + CS$$

Acceptable limits of the S–D_{tol} relationship were defined by the standard error (SE) of the estimate: CS <2% and D' < 10% (Ferguson et al., 2013). VO_2 and HR were measured when subjects were considered close to exercise intolerance, while [La^-] was measured 2 min post-cessation, as described above.

Time to exhaustion

As an external test of exercise capacity and performance within the S–D_{tol} relationship and as such of the validity of the S–D_{tol} relationship after it had been individually established, time to exhaustion was empirically assessed at the extremes of the S–D_{tol} relationship, by constant running speeds corresponding to (1) CS, and (2) 110% of that eliciting VO_{2max}, individualised to each subject as representations of speeds in the extreme lower and upper ends of the S–D_{tol} relationship. VO_2, HR, and [La^-] were measured close to exercise intolerance, as described above. As a final test, time to exhaustion trials were repeated after supplementation of 900 mg/d BC (315 mg/d anthocyanin), administered as described above.

Statistics

The Shapiro–Wilk test confirmed normal distribution. BC versus placebo effects were assessed by paired samples *t*-tests, and where applicable, one-way analysis of variance (ANOVA), while serial recordings were assessed by repeated measures general linear model; Scheffe post-hoc tests identified effects. Statistical significance was assessed by $p < .05$ and the 95% confidence interval of the difference, whereas Cohen's *d* reported effect size. Data are expressed as means ± standard deviation (SD). Statistical analysis was conducted using SPSS version 26 (IBM, Armonk, NY).

Results

Subject characteristics

Subject characteristics are presented in Table I, indicating a healthy, young, recreationally active male cohort. Body composition, fitness and blood pressure were within a healthy range and did not change with BC extract supplementation; systolic and diastolic blood pressures after BC supplementation were 121.4 ± 5.5mmHg ($p > .05$ [−3.1–2.5] vs placebo) and 82.2 ± 5.9mmHg, respectively ($p > .05$ [−1.4–2.7] vs placebo).

Speed–tolerable duration

From the constant speed-tests, the S–D_{tol} relationship was plotted for each subject, with tolerable duration well described as a hyperbolic function of speed (example traces in Figure 1(A)) and CS and D' calculated after linear S–D_{tol}^{-1} transformation (example traces in Figure 1(B)), with the SE well within the acceptable limits of <2% for CS (range 0.4–1.6%) and <10% for D' (range 2.0–8.3%).

BC extract supplementation effects on CS and D' are represented in Figures 1(C,D), respectively, with no statistical differences occurring between BC and placebo for either CS ($p > .05$ [−0.3–0.1]) or D' ($p > .05$ [−39.1–124.6]). Further analysis however indicated a weak, but statistically insignificant trend toward D' increasing in 60% of subjects by a magnitude of 10–40% ($p = .08$, Cohen's $d = 0.17$ in those that indicated a trend), suggesting a possibility that certain cohorts may potentially gain a small benefit from BC supplementation. Order of intake of BC extract or placebo did not correlate with the trend toward increased D' in the aforementioned 60% of subjects ($r^2 = 0.106$, $p > .05$). A similar trend for CS was not observed.

VO_2 and HR measured when subjects were considered close to exercise intolerance during each constant speed-test confirmed volitional exhaustion and attainment of VO_{2max} and HR_{max} in each test, as expected and required of a valid test (Burnley & Jones, 2018; Ferguson et al., 2013; Poole et al., 2016). Comparisons between VO_2 measured during the constant speed-tests and the incremental exercise test under both placebo and BC conditions demonstrate that no differences occurred ($p = .981$; Figure 1(E)). Similar effects were reproduced when measuring HR during incremental exercise and constant speed-tests for either of placebo (incremental exercise test 194 ± 6 bpm vs constant speed-tests 186 ± 8 bpm, $p > .05$) or BC (incremental exercise test 191 ± 5 bpm vs constant speed-tests 187 ± 6 bpm, $p > .05$) conditions, with no differences occurring (data not shown); further confirming that volitional exhaustion and exercise intolerance was objectively achieved during each test.

Measurements of post-exercise blood [La^-] showed a decline from fastest to slowest constant speed-tests (range 11.5–4.4 mM), but with no differences occurring between placebo and BC ($p > .05$ [−0.7–0.7]; data not shown).

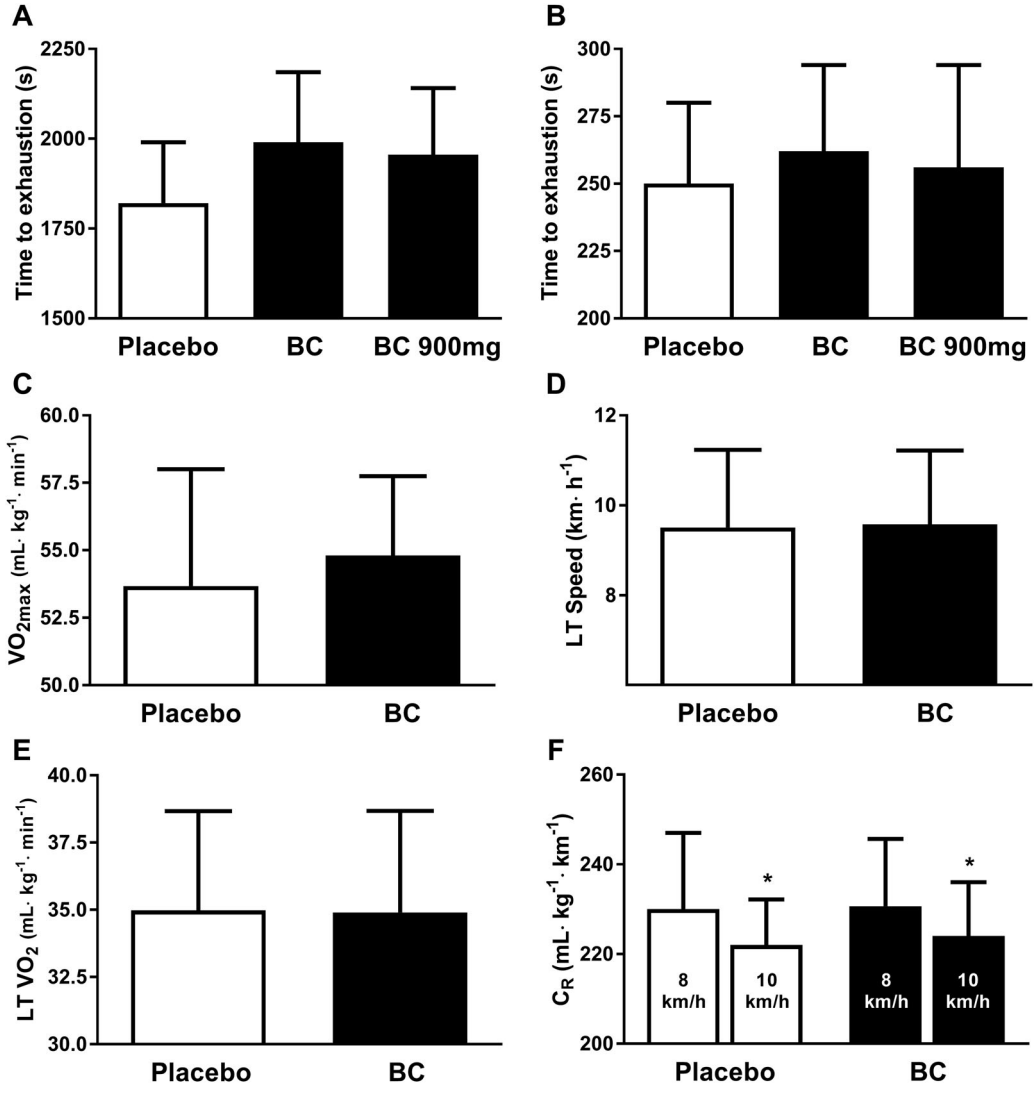

Figure 2. Time to exhaustion during fixed running speeds after placebo or supplementation with anthocyanin-rich blackcurrant extract (BC) at normal dose (300 mg New Zealand Blackcurrant Extract, 105 mg anthocyanin) or high dose (900 mg New Zealand Blackcurrant Extract, 315 mg anthocyanin): (A) at critical speed (CS); (B) at 110% of maximal oxygen uptake (VO_{2max}). Aerobic capacity measured by incremental exercise testing; (C) VO_{2max}; (D) running speed at lactate threshold (LT); (E) oxygen uptake (VO_2) at LT; (F) running economy (C_R) at 8 and 10 km/h. No differences occurred between placebo and BC ($p > .05$). Statistically significant difference 8 km/h vs 10 km/h: $^*p < .05$.

Time to exhaustion

As an empirical test of the effect of BC extract supplementation on the S–D_{tol} relationship, we measured time to exhaustion at two fixed speeds, one corresponding to the calculated CS and another corresponding to an exercise intensity that would elicit exercise intolerance within the steeper, upper end of the hyperbolic S–D_{tol} relationship.

At a running speed corresponding to CS (Figure 2 (A)), time to exhaustion ranged 25:40–38:04 min:sec (1540–2284 s), whereas at a running speed within the steep upper section of the S–D_{tol} relationship, a speed corresponding to 110% of that eliciting VO_{2max} (Figure 2(B)), time to exhaustion ranged 3:05–6:07 min:sec (185–367 s). However, time to exhaustion did not differ between placebo and BC supplementation at either running speed (CS-speed $p > .05$ [−82.1–121.8] and 110% of VO_{2max}-speed $p>0.05$ [−13.8–28.1]), though further analysis indicated a weak, but statistically insignificant trend toward time to exhaustion increasing in 60% of subjects by a magnitude of 10–20% ($p = 0.09$, Cohen's $d = 0.59$ and 0.42 in those that indicated a trend for CS-speed and 110% of VO_{2max}-speed, respectively), in line with the previous suggestion that certain cohorts may potentially benefit from BC supplementation. As for D', order effects BC extract versus placebo did not correlate with the trend toward time to exhaustion ($r^2 = -0.065$, $p > .05$). As a final

assessment, we repeated the time to exhaustion trials, but after supplementation with 900 mg BC (3x normal dose). This did not affect time to exhaustion performance (Figure 2(A,B)).

In line with the constant speed-tests used to establish the S–D_{tol} relationship, measurements of VO_2 and HR confirmed that volitional exhaustion and exercise intolerance was objectively achieved at each test by attainment of VO_{2max} and HR_{max} (data not shown). Post-exercise blood [La^-] also reached the expected elevated levels (range 5.9–15.5 mM); however, no differences occurred between placebo and BC ($p > .05$ [−1.4–3.1]; data not shown).

Aerobic capacity, lactate threshold and running economy

Next, we compared results from the incremental exercise tests. Neither of VO_{2max} (Table I and Figure 2(C); $p > .05$ [−0.9–2.1]) or HR_{max} (Table I; BC supplementation 191 ± 5bpm; $p > 0.05$ [−4–4]) differed significantly between BC extract supplementation and placebo.

LT, measured as running speed (Figure 2(D)) at the point of blood La^- accumulation, did not significantly differ between BC extract supplementation and placebo ($p > .05$ [−0.6–0.6]). LT speed was notably below CS (~9.5 vs ~12 km/h; $p < .05$ [2.0–2.9]) under both placebo and BC conditions. LT assessed by VO_2 (Figure 2(E)) did also not differ significantly between BC supplementation and placebo ($p > .05$ [−1.8–1.6]), and similarly, nor did LT assessed by HR ($p > .05$ [−4–3]; data not shown). VO_2 at LT corresponded to 63 and 64% of VO_{2max} after placebo and BC supplementation, respectively, whereas HR at LT corresponded to 76% and 74% of HR_{max} after placebo and BC supplementation. As a result of neither speed or VO_2 at LT differing between placebo and BC supplementation (Figure 2(D,E)), C_R at LT, expressed as oxygen cost of running ($mL \cdot kg^{-1} \cdot km^{-1}$), did also not differ between placebo and BC supplementation ($p > .05$ [−4.8–6.5]).

To further study C_R, we recorded steady-state oxygen cost at fixed submaximal running speeds 8 and 10 km/h, corresponding to < LT and > LT (LT 9.5 ± 1.6 km/h), respectively (Figure 2(F)). This showed that C_R improved by 3% ($p < .05$ [−8.3–5.8]) when increasing running speed from 8 km/h to 10 km/h, but did not differ between placebo and BC extract supplementation ($p > .05$ [−4.1–4.4]).

Energy expenditure and substrate utilisation

Energy expenditure and substrate utilisation were assessed during steady-state submaximal exercise conditions at an exercise intensity corresponding to LT (Table II). This showed that no differences occurred between placebo and BC extract supplementation. Intensities above LT and within the S–D_{tol} relationship were not assessed, since they are not performed during steady-state conditions.

Discussion

This is the first study to assess the effect of daily supplementation with anthocyanin-rich BC extract on the S–D_{tol} relationship and its parameters CS and *D*' during running. Previous studies had indicated that BC supplementation may improve high-intensity exercise capacity and performance (Cook et al., 2015; Godwin et al., 2017; Murphy et al., 2017; Perkins et al., 2015; Willems et al., 2016); exercise that may at least partly be determined by S–D_{tol} (Burnley & Jones, 2018; Ferguson et al., 2013; Poole et al., 2016). However, in our study, we found no such effect, as our results indicate that the previously reported improvements to high-intensity exercise do not arise via improvements to CS, *D*', and the S–D_{tol} relationship. Although we did note a trend toward increased *D*' and time to exhaustion in a cohort of our subjects, but these were weak trends that did not reach statistical significance and the effect sizes were small to medium, but variable. Intriguingly though, these were not order effects and are in line with previous findings (Murphy et al., 2017) of potential cohort effects, but neither our nor previous studies have been able to identify characteristics within those cohorts sufficient to prospectively target responders vs non-responders for BC supplementation, although it has been noted that benefits of BC supplementation may depend on training history (Godwin et al., 2017) and ethnicity (Willems et al., 2018).

Our main conclusion relies on establishment of the S–D_{tol} relationship. A criticism of the concept may be that it is a computation and not a measurement *per se*, albeit it is based upon at least 4 separate constant speed-tests (Ferguson et al., 2013). We however note that all were deemed valid by close mathematical and physiologic scrutiny of the limits of acceptable error (Ferguson et al., 2013) and by attainment of VO_{2max} in each separate test due to the slow component of VO_2 that inevitably occurs above CS (Burnley & Jones, 2018; Ferguson et al., 2013; Poole et al., 2016). Regardless of this, we also empirically measured actual performance within the running speed-intensity range covered by the S–D_{tol} relationship by subsequent time to exhaustion tests designed to correspond to (1) CS and (2) running speeds in the steep part of the S–D_{tol} relationship well above

Table II. Energy expenditure and substrate utilisation during exercise at lactate threshold ($n = 15$)

	Placebo	BC
Energy expenditure ($kJ \cdot min^{-1}$)	58.64 ± 5.14	56.2 ± 6.55
CH oxidation ($g \cdot min^{-1}$)	2.26 ± 0.61	2.15 ± 0.41
Fat oxidation ($g \cdot min^{-1}$)	0.59 ± 0.26	0.56 ± 0.44

Notes: Data are mean ± standard deviation (SD). BC: supplementation with anthocyanin-rich blackcurrant extract; CH: carbohydrate; No differences occurred between placebo and BC ($p > .05$).

CS, where tolerable exercise duration deteriorates rapidly with increasing speeds. Similar to our other results, BC supplementation did not affect time to exhaustion performance, including after subjects received 3x doses of BC supplementation. As such, we conclude that BC supplementation does not alter $S–D_{tol}$ during running in any predictable manner, and improvements to exercise capacity and performance must therefore originate elsewhere.

High-intensity endurance exercise capacity and performance is also physiologically determined by VO_{2max}, C_R and fractional utilisation of VO_{2max} during submaximal running, and LT (Bassett & Howley, 2000); thus, we also included those in our investigation. However, BC supplementation did not affect any of those parameters. This was an expected finding though, based upon previous studies (Cook & Willems, 2019; Perkins et al., 2015; Willems et al., 2018; Willems et al., 2019).

In a departure from comparable previous studies (Cook et al., 2015; Strauss et al., 2018) and studies finding upregulation of lipid metabolism genes after cellular exposure to anthocyanins (Tsuda, Ueno, Kojo, Yoshikawa, & Osawa, 2005), we found no effect of BC supplementation on substrate utilisation and fat oxidation during submaximal exercise corresponding to ~65% of VO_{2max}. It would have been interesting to assess fat oxidation during higher-intensity workloads within the $S–D_{tol}$ relationship, but this was not feasible in our study since the measurement arrives from steady-state gas exchange recordings, conditions which due to the VO_2 slow component were not present at these intensities (Burnley & Jones, 2018; Ferguson et al., 2013; Poole et al., 2016). Fat oxidation is however not substantial during high-intensity exercise, whereas in contrast carbohydrate oxidation is (Achten, Gleeson, & Jeukendrup, 2002), but for the same reason could not be estimated during high-intensity exercise.

We acknowledge that in our study, supplementary BC administration differed from most studies (Cook et al., 2015; Cook et al., 2017a; Godwin et al., 2017; Murphy et al., 2017; Perkins et al., 2015; Strauss et al., 2018; Willems et al., 2016; Willems et al., 2018; Willems et al., 2019) with respect to pre-loading, but not dose (Cook et al., 2015; Cook et al., 2017a; Murphy et al., 2017; Perkins et al., 2015; Willems et al., 2016), daily time of intake, time prior to testing, or intake with other food. Specifically, most studies have applied a 7-day phase-in period for supplementation, whereas in contrast we applied a 1-day phase-in period. This is because very recent studies (Alvarez-Suarez et al., 2014; Hurst et al., 2019; Krga & Milenkovic, 2019) have shown that plasma concentration of anthocyanins peaks at 2 h and clears within 6–12 h post-consumption after intake in the 0.8–3.2 mg/kg range, which is a range that also covers our administration of 1.3–1.5 mg/kg (105 mg/d) anthocyanin, and which therefore suggests that anthocyanin bioavailability is unlikely to differ between the reported short-term 1–7-day pre-loading regimens. However, it remains possible that long-term anthocyanin metabolite build-up may affect the outcome on specific parameters, such as the observation that 5-week daily preloading alleviated markers of post-exercise oxidative stress and inflammation (Hurst et al., 2020). Nonetheless, administration in our study was at the low end vs other studies with respect to pre-loading and dosage and may have accounted for the lack of significant beneficial effects. However, as noted above, we administered a similar daily dose as most studies (Cook et al., 2015; Cook et al., 2017a; Murphy et al., 2017; Perkins et al., 2015; Willems et al., 2016) that have reported a beneficial effect, and furthermore, increasing dosage to 900 mg/d BC (315 mg/d anthocyanin) did not cause an effect, which is comparable to previous work reporting no dose-dependent effects on VO_2 or work economy (Cook, Myers, Gault, Edwards, & Willems, 2017b), albeit dose-dependent cardiovascular (Cook et al., 2017a) and substrate oxidation (Krga & Milenkovic, 2019) effects have been noted during supine rest and sub-maximal exercise. It also remains possible that mode of intake may affect the outcome, but juice (Hutchison et al., 2016; Lima et al., 2019) or dry powder-based (Willems et al., 2015) supplements have not proven more successful. Finally, and as detailed above, we sought to minimise confounding factors, but some day-to-day variation in e.g. subject motivation or nutrition and energy

intake consumed during breakfast cannot be excluded; but if they were present, they may have added ecological validity.

As such, we return to the question: does supplementary BC consumption enhance exercise capacity and performance and should it be promoted as an ergogenic aid? Our study cannot find evidence of BC supplementation enhancing high-intensity exercise capacity and performance or enhancing the underlying physiologic parameters that govern high-intensity exercise, but nor do we find evidence of any detrimental effects. Thus, we cannot warrant the use of BC supplementation as an ergogenic aid, but we acknowledge that some (Cook et al., 2015; Cook et al., 2017a; Godwin et al., 2017; Hutchison et al., 2016; Lima et al., 2019; Murphy et al., 2017; Perkins et al., 2015; Strauss et al., 2018; Willems et al., 2014; Willems et al., 2015; Willems et al., 2016), but not all (Willems et al., 2015, 2018, 2019) studies have indicated a positive ergogenic effect. Reasons for this discrepancy remain unknown, but it is possible that administration of BC supplementation must be concurrent to a high training load before a benefit may be realised, given that BC supplementation in some studies has facilitated recovery between exercise sessions (Godwin et al., 2017; Murphy et al., 2017; Willems et al., 2016), possibly linked to enhanced La^- handling with reduced production and/or increased clearance (Willems et al., 2015) and tolerance (Perkins et al., 2015), reduced oxidative stress and inflammatory immune reactions (Ehala et al., 2005; Hurst et al., 2019; Hurst et al., 2020; Hutchison et al., 2016; Lyall et al., 2009; Wallace, 2011), and reduced muscle damage (Lima et al., 2019) following high-intensity exercise.

Conclusion

We could not detect any beneficial or otherwise effect of daily anthocyanin-rich commercially available BC extract supplementation to the $S–D_{tol}$ relationship during running, including its parameters CS and *D*' or associated time to exhaustion trials as empirical tests of the $S–D_{tol}$ relationship, and nor could we detect any effect on VO_{2max}, C_R and fractional utilisation of VO_{2max} during submaximal running, or LT. Hence, BC extract supplementation in our study did not demonstrate benefit to high-intensity exercise capacity or performance.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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