New Zealand Blackcurrant Extract Improves High-intensity Intermittent Running

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Abstract

We examined the effect of New Zealand blackcurrant (NZBC) extract on high-intensity intermittent running and post-running lactate responses. Thirteen active males (age: 25±4 yrs, height: 1.82±0.07 m, body mass: 81±14 kg, $\dot{V}O_{2max}$: 56±4 mL·kg^{-1}·min^{-1}, $_{v}\dot{V}O_{2max}$: 17.6±0.8 km·h^{-1}) performed a treadmill running protocol to exhaustion, which consisted of stages with 6x19 s of sprints with 15 s of low-intensity running between sprints. Inter-stage rest time was 1 minute and stages were repeated with increasing sprint speeds. Subjects consumed capsuled NZBC extract (300 mg·day^{-1} CurraNZ™; containing 105 mg anthocyanin) or placebo for 7 days (double blind, randomised, cross-over design, wash-out at least 14 days). Blood lactate was collected for 30 min post-exhaustion. NZBC increased total running distance by 10.6% (NZBC: 4282±833 m, placebo: 3871±622 m, *P*=0.02), with the distance during sprints increased by 10.8% (*P*=0.02). Heart rate, oxygen uptake, lactate and rating of perceived exertion were not different between conditions for the first 4 stages completed by all subjects. At exhaustion, blood lactate tended to be higher for NZBC (NZBC: 6.01±1.07 mmol·L^{-1}, placebo: 5.22±1.52 mmol·L^{-1}, *P*=0.07). There was a trend for larger changes in lactate following 15 min (NZBC: -2.89±0.51 mmol·L^{-1}, placebo: -2.46±0.39 mmol·L^{-1}, *P*=0.07) of passive recovery. New Zealand blackcurrant extract (CurraNZ™) may enhance performance in sports characterised by high-intensity intermittent exercise as greater distances were covered with repeated sprints, there was higher lactate at exhaustion, and larger changes in lactate during early recovery after repeated sprints to exhaustion.

Key words: anthocyanin, repeated sprints, recovery

INTRODUCTION

Supplement intake among athletes is common to support training practice and enhance sports performance. Research on ergogenic aids has recently shifted attention towards an understanding of functional food ingredients to enhance both health and sports performance (Bell et al., 2014; Shipp & Abdel-Aal, 2010). For example, anthocyanin-containing products have been associated with health benefits such as prevention and suppression of obesity and diabetes (Prior et al., 2008; Sasaki et al., 2007), reduced risk for cardiovascular disease (Wallace, 2011), suppression of inflammatory pathways associated with cancer pathogenesis (Prasad et al., 2010), and enhanced brain function (Spencer, 2010). Anthocyanin-induced effects may be attributed to an altered endothelial function (Speciale et al., 2014), potentially by up-regulation of the endothelial nitric oxide synthase (eNOS), an enzyme involved in the production of endogenous nitric oxide (NO), and providing as such a mechanism for enhanced peripheral blood flow to exercising muscles via relaxation of vascular smooth muscle cells and vasodilation of blood vessels (Suhr et al., 2013). Evidence in support was provided by Ziberna et al (2013) who demonstrated anthocyanin-induced vasorelaxation and vasodilation in the thoracic aortic rings of male Wistar rats. Furthermore, enhanced peripheral blood flow by 22% in the forearm of humans and reduced fatigue during typing was shown three hours after blackcurrant concentrate intake (Matsumoto et al., 2005), and increases in flow-mediated dilation by intake of purified anthocyanin or polyphenols in healthy populations (Khan et al., 2014; Rodriguez-Mateos et al., 2013).

Recovery from exercise is influenced by peripheral circulation and venous return (Bieuzen et al., 2012), thus blackcurrant intake may promote post-exercise recovery from high-intensity exercise. The effect of blackcurrant on blood flow may even enhance the performance of high-intensity exercise such as repeated sprints, common in certain team sports. In those sports (e.g. soccer), approximately 70-85% of match play may consist of low and moderate intensity activities (Bangsbo et al., 2006), with remaining play time

characterised by abrupt and repeated changes in intensity. Fatigue during high-intensity intermittent exercise is associated with phosphocreatine (PCr) degradation and metabolite accumulation (Glaister, 2005). Thus, interventions that blunt PCr degradation and/or reduce metabolite accumulation will be advantageous for high-intensity intermittent exercise performance (McMahon & Jenkins, 2002). The importance of blood flow and corresponding muscle oxygen delivery in PCr resynthesis is recognized (Sahlin et al., 1979), with increased muscle oxygen delivery also shown to reduce PCr degradation during plantar flexion exercise to exhaustion (Hogan et al., 1999). It needs to be recognized, however, that the exercise model in the present study is intermittent running with high intensity to exhaustion. Nevertheless, increased muscle oxygen availability may enhance PCr resynthesis during the recovery periods of intermittent exercise (Billaut & Buchheit, 2013). Furthermore, blood flow, and potentially the manner in which it is distributed, may contribute towards lactate clearance, primarily via oxidation (approximately 70–80%) and gluconeogenesis (approximately 20–30%) (Brooks, 2007). Thus, the effect of blackcurrant intake on peripheral blood flow may help maintain PCr stores and decrease metabolite accumulation; blackcurrant may delay the onset of fatigue, enhance the performance of repeated sprints and improve post-exercise recovery.

Therefore, we examined the effects of New Zealand blackcurrant extract on physiological responses and performance of high-intensity intermittent running to volitional exhaustion. Our primary hypothesis was that blackcurrant intake would enhance running performance, measured by distance covered during repeated sprints. It was also hypothesised that recovery from repeated sprints to exhaustion, measured by blood lactate levels, would be improved by New Zealand blackcurrant intake.

METHODS

Participants

Thirteen healthy male participants were recruited (mean±SD, age: 25±4 years, mass: 81±14 kg, height: 1.82±0.07 m, $\dot{V}O_{2max}$: 56±4 ml·kg·min^{-1}, $_{v}\dot{V}O_{2max}$: 17.6±0.8 km·h^{-1}). Participants were recreationally active with experience in sports with high-intensity intermittent exercise and most familiar with treadmill running. Participants refrained from additional supplementation during the study, provided informed written consent and did not receive payment. The study was approved by the University of Chichester Research Ethics Committee and conformed to the Declaration of Helsinki.

Experimental Design

The study comprised of three sessions within five weeks. In the first visit, participants performed a rapid ramp test to exhaustion to determine $\dot{V}O_{2max}$, followed by a verification phase to confirm $\dot{V}O_{2max}$ (Midgley & Carroll, 2009). Subsequently, participants were familiarized with the high-intensity, intermittent treadmill based running test. Participants were randomly assigned in a double blind, cross-over design to receive seven days of NZBC supplementation or placebo. During the experimental visits (testing sessions two and three) participants performed a continuous/intermittent warm up protocol before completing the running test. Experimental visits were separated by a period of at least 21 days and no more than 45 days, allowing a 14 day wash-out period and a second supplementation period of 7 days.

All sessions were conducted in laboratory conditions (17-19°C and 60–75% humidity) and the running test was carried out on a motorised treadmill (H/P/COSMOS, Groningen, Netherlands) at a 1% gradient. Expired air was collected via a breath-by-breath gas analyser (Jaegar Oxycon Pro, Cardinal Health, Basingstoke, UK). This system was calibrated with gases of known concentration, and the tube flowmeter was calibrated by a 3-L syringe for each session. All blood samples were analysed within 30 seconds of collection (YSI 2300,

Analytical Technologies, Farnborough, Hants, UK). Participants recorded their food intake and physical activity in the 24 hour preceding the first experimental visit and to replicate this in the 24 hours preceding the subsequent visit. Participants refrained from caffeine and alcohol 24 hours before each session and abstained from vigorous exercise during this period. Experimental trials were conducted at the same time of day (±2 hours) to limit any circadian rhythm variation.

Experimental Procedures

Rapid Ramp $\dot{V}O_{2max}$ Verification Test

The test commenced at an individually determined speed and increased by 0.1 $km \cdot h^{-1}$ every 5 seconds until exhaustion. $\dot{V}O_{2max}$ was taken as the highest 15-breath average value attained prior to exhaustion. Ten minutes after the termination of the rapid ramp test, a verification square wave test to exhaustion was conducted. Running speeds for the verification protocol were determined by the speed achieved at $\dot{V}O_{2max}$ (100% $_{v}\dot{V}O_{2max}$) during the rapid ramp protocol. The verification square wave test commenced with a 3 minute period at 50% $_{v}\dot{V}O_{2max}$, before an abrupt increase to 100% $_{v}\dot{V}O_{2max}$. Participants were given no temporal feedback but were verbally encouraged to continue until volitional exhaustion during both tests. Attainment of a true $\dot{V}O_{2max}$ was confirmed by consistent peak $\dot{V}O_2$ values in the rapid ramp and verification protocols (Midgley & Carroll, 2009).

High-Intensity Intermittent Treadmill Running Test

Prior to the running test, participants completed a warm up protocol. This protocol comprised of a five minute continuous stage at 50% $_{v}\dot{V}O_{2max}$, followed by a three minute alternate walk (30% $_{v}\dot{V}O_{2max}$) and run (60% $_{v}\dot{V}O_{2max}$), with speeds alternating every 15 seconds. Upon completion of the warm up, participants had five minutes for self-selected stretching after which a pre-test fingertip capillary blood sample was taken for lactate.

The running protocol involved 3 phases and was adapted from the NIE Intermittent High-Intensity test (Mukherjee & Chia, 2013). The first phase consisted of five minutes running at 60% $_{v}\dot{V}O_{2max}$. The second phase comprised of seven stages, with each stage lasting a total of 204 seconds (six repeated sprints lasting 19 seconds interspersed with active recovery bouts (always at 50% $_{v}\dot{V}O_{2max}$) lasting 15 seconds) and interspersed with 60 seconds of passive recovery between the stages in which rating of perceived exertion (RPE) was recorded and a fingertip blood sample was taken for lactate. The speed for the sprints was calculated by a percentage of $_{v}\dot{V}O_{2max}$ with stage one being set at 80% $_{v}\dot{V}O_{2max}$. Running speed of the sprints in each stage was then increased by 5% $_{v}\dot{V}O_{2max}$ per each stage, up to 110% $_{v}\dot{V}O_{2max}$ (stage 6). Thereafter, in phase three (≥ stage 7), the speed increased by 2.5% $_{v}\dot{V}O_{2max}$ per stage until volitional exhaustion. The treadmill required ~2-4 seconds to accelerate/decelerate between speeds and reach the set velocity. Sprint speeds were between 11.5 ± 5.7 km·h^{-1} (first sprint) and 18.0 ± 1.18 km·h^{-1} (final sprint). Active recovery speeds were 7.2 ± 3.6 km·h^{-1}. During the test, participants were informed of the beginning and end of a sprint and received verbal encouragement to perform at maximum effort in all testing sessions. Participants did not receive feedback on the distance covered, number of sprints and stage number.

During the running test, expired air was collected via online breath-by-breath system (Jaegar Oxycon Pro, Cardinal Health, Basingstoke, UK). Heart rate (Consultancy RS800, Polar Electro UK Ltd, Warwick, UK) was recorded during each exercise protocol, with participants also reporting ratings of perceived exertion (RPE, 15-point scale) between each stage. Upon completion of the running test, recovery was monitored with fingertip blood samples taken at 1, 2, 3, 4, 5, 10, 15 and 30 minutes.

Supplementation

Participants received seven days of NZBC supplementation [105 mg anthocyanin (delphinidin-3-rutinoside 35-50%, delphinidin-3-glucoside 5%-20%, cyanidin-3-rutinoside

30-45%, cyanidin-3-glucoside 3-10%)] per dose of 300 mg CurraNZ™; administered as one capsule per day; CurraNZ™, Health Currancy Ltd, Surrey, UK) or PL (300 mg microcrystalline cellulose M102; administered as one capsule per day). The optimal dosing strategy for New Zealand blackcurrant powder is not known and the administered dose was according to manufacturer's instructions. However, studies on demonstrating the effectiveness of berry juices have used a multiple day dosing strategy in before exercise testing (e.g. 8 days: Bowtell et al., 2011; 6 days: Howatson et al., 2010). On the morning of the final day of supplementation, subjects consumed their last supplement three hours prior to testing. Participants were also asked to arrive in a fully hydrated state and consume a light breakfast (i.e. toast with jam or small bowl of cereal) ≥ 2 hours prior to testing. We recognise a limitation that familiarization for the repeated sprint protocol occurred after maximum oxygen uptake testing but the familiarization performance was only 3 sprints lower (i.e. 29 ± 4) than performance during placebo testing.

Data Analysis

Oxygen uptake

Breath-by-breath oxygen uptake ($\dot{V}O_2$) data was examined to exclude errant breaths, and values more than four standard deviations from the local mean were removed. $\dot{V}O_2$ data was then averaged for each stage, so that total analysed time was 204s (6 × (19s sprint + the following 15s recovery periods)). This analysis was conducted up to the completion of stage 4 for all participants under both conditions because stage 4 was reached by all participants. $\dot{V}O_2$ data of 4 participants was excluded due to technical problems.

Statistical Analysis

Differences between NZBC and PL in total distance covered, distance covered during high-intensity running, distance covered during active recovery bouts and number of sprints during the running test were analysed using paired samples *t*-tests. A two-way repeated measures

ANOVA was used to analyse differences between groups and over time for 1) $\dot{V}O_2$, blood lactate, HR, and RPE) up to the completion of stage 4, due to participant drop out commencing after this stage, 2) absolute blood lactate values and 3) changes in blood lactate values during passive recovery. Significance for between group differences, time effects and interaction effects were analysed with post hoc paired samples *t*-tests. A priori power analysis showed a sample size of 12 would allow detection of a 9% difference in sprint distance with a high statistical power ($1 - \beta = 0.80$: $0.05 = \alpha$ level). Statistical significance was accepted at $P<0.05$. Interpretation of $0.05>P \leq 0.1$ was according to guidelines by Curran-Everett & Benos (2004). Data are presented as mean ± SD unless stated otherwise. All statistical procedures were conducted using statistical package SPSS v 20.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Running performance

Participants were able to increase the number of sprints from 32 ± 4 (PL) to 35 ± 6 (NZBC) (P=0.020). The total distance that was covered during the high-intensity intermittent running protocol was 10.6% greater with intake of NZBC (4282 ± 833 m) compared to PL (3871 ± 622 m) (P=0.023). The increase in total distance was therefore due to an enhanced ability to cover more distance during the repeated sprints by 10.8% (NZBC: 2849 ± 570 m, PL: 2572 ± 421 m, P=0.024) (Figure 1) and during active recovery running by 10.3% (NZBC: 1433 ± 264 m, PL: 1299 ± 203 m, P=0.023).

Physiological and perceptual responses

In both conditions, there was an increase in heart rate, oxygen uptake, RPE (Table 1) and blood lactate (Figure 2) (all $P<0.05$) during the high-intensity intermittent running protocol. However, there were no differences between conditions for heart rate (P=0.33), oxygen

uptake (P=0.37), blood lactate (P=0.81) and RPE (P=0.79) in each of the first 4 stages that were completed by all participants, and no interaction effect (i.e. heart rate (P=0.52), oxygen uptake (P=0.64), blood lactate (P=0.47) and RPE (P=0.12). At exhaustion, there was a trend for blood lactate to be higher by 15% (P=0.07) (Figure 2) with NZBC intake with 9 out of 13 subjects having higher values, suggesting that with NZBC intake higher blood lactate values were achieved.

Post-exercise recovery of lactate

During passive recovery, absolute lactate values became lower over time (P<0.05) in both conditions with a trend for an effect of the supplementation (P=0.07) to have larger absolute blood lactate after 1 (P = 0.07), 2 (P = 0.09), 3(P = 0.08), 4 (P = 0.07), 10(P = 0.07) and 30 minutes (P = 0.07) (Figure 3). There was no interaction effect (P=0.94). There was a trend for larger changes in blood lactate following NZBC intake after 15 minutes (NZBC: -2.89±0.51 mmol·L^{-1}, PL: -2.46±0.39 mmol·L^{-1}, P = 0.07).

DISCUSSION

This study provides evidence for the ergogenic potential of New Zealand blackcurrant extract on high-intensity exercise performance; repeated sprint distance in a high-intensity intermittent running test was improved by 10.8%. This improvement occurred without alterations in heart rate, oxygen uptake, blood lactate and RPE values in the first 24 sprints that were completed by all participants compared to placebo. We also observed a trend to reach exhaustion from repeated sprints with higher blood lactate. In addition, following exhaustion, there was a trend to have larger reductions in blood lactate during the 30-min of passive recovery. However, larger changes in blood lactate during recovery with New Zealand blackcurrant may be due to the mass action effect, i.e. faster rates of removal are due to higher lactate values at the start of the recovery.

Potential mechanisms for New Zealand blackcurrant extract on performance

Peripheral muscle fatigue from repeated high-intensity exercise may involve effects of accumulation of metabolites and by-products of metabolic pathways, changes in ionic concentrations and reduced energy supply (Girard et al., 2011). High-intensity repeated exercise lowers intracellular muscle pH (i.e. acidosis). It also increases extracellular potassium and intracellular sodium and chloride concentrations (McKenna et al., 2008) that cause reduced muscle excitability along the muscle and t-tubular membranes. Intracellular acidosis was also suggested to be able to modulate the voltage-gated chloride channel potentially postponing reductions in muscle excitability (Pedersen et al., 2004). Therefore, New Zealand blackcurrant may postpone peripheral muscle fatigue by allowing elevated levels of intracellular acidosis. However, future work should address whether elevated levels of intracellular acidosis occurred with New Zealand blackcurrant intake during high-intensity running to exhaustion as higher lactate values may only suggest this to be the case. Elevated levels of acidosis may offset the negative consequences of disbalanced ion concentrations along the muscle and t-tubular membranes on muscle excitability. In addition, blackcurrant fruit extract is known to have antioxidant activity (Bonarska-Kujawa et al., 2014). During high-intensity exercise, the oxidative stress and associated production of reactive oxygen species is counteracted by antioxidants. Reactive oxygen species may have a negative effect on the sodium-potassium pump (McKenna et al., 2006) and calcium handling by the sarcoplasmic reticulum (Favero, 1999) causing fatigue. It is therefore likely that the fatigue process during high-intensity exercise linked with the production of reactive oxygen species (Morales-Alamo and Calbet, 2014) can be influenced by blackcurrant intake. For example, acute oral intake of the antioxidant N-acetylcysteine improved performance supplementation on the Yo-Yo Intermittent Recovery Test Level 1 (Cobley et al., 2011), potentially by

attenuation of the decline in the activity of the sodium-potassium pump (McKenna et al., 2006) and postponing fatigue.

Several studies provided evidence for an effect of polyphenols on vascular function (Khan et al., 2014; Rodriguez-Mateos et al., 2013). Peripheral blood flow was increased by 22% in the forearm in rest with intake of blackcurrant concentrate (1.84 mg anthocyanins per kg body weight) (Matsumoto et al., 2005). In the present study, the New Zealand blackcurrant product that was used is an anthocyanin-rich extract containing 105 mg of anthocyanins per capsule. Increased peripheral blood flow in leg muscles may have occurred in the present study between the stages (i.e. participants in rest), and allowing higher phosphocreatine resynthesis and reduced metabolite accumulation. In addition, the improved recovery as characterized by larger changes in blood lactate may also be due to increased peripheral blood flow enabling lactate transport to other tissues for oxidation.

Anthocyanins and bioavailability

Anthocyanins are rapidly absorbed, reaching peak levels in the blood within 1 to 2 hours (Matsumoto et al., 2005; Stoner et al., 2005) with metabolites peaking later and elimination of anthocyanins and metabolites completed after 48 hrs (Czank et al., 2013). We were not able to quantify the bioavailability of anthocyanins and metabolites in the blood in the present study. However, although our participants took the New Zealand blackcurrant for 7 days, the last intake was 3 hours before attending the exercise session. The optimal dosing strategy of New Zealand blackcurrant is not known, therefore the dose and duration of administration was according manufacturer's guidelines. An understanding of the ergogenic potential and mechanisms of action requires an understanding of anthocyanin bioavailability, taking into account factors affecting absorption, metabolism, distribution and elimination.

New Zealand blackcurrant extract and high-intensity intermittent running

297 Our treadmill running protocol was adapted from an intermittent treadmill running
298 test by Mukherjee and Chia (2013) to examine running capability in soccer players; this test
299 was shown to be a reliable (ICC, 0.98; CV, 2.1%) measure of high-intensity intermittent
300 running capability in soccer players. Performance of the high-intensity intermittent test by
301 Mukherjee and Chia (2013) correlated strongly (r=0.68-0.77) with performance on the YoYo
302 IR2 test (Krustrup et al., 2006). Performance in the YoYo IR2 test correlates with the
303 amount of intense exercise performed by team sport players (Bangsbo et al., 2008).
304 Therefore, NZBC extract may be able to enhance performance in sports with high-intensity
305 intermittent running. In our study, the total distance in our running protocol consisting of
306 repeated sprints and active recovery running was increased by 10.6% (411 m) after
307 supplementation. Because laboratory based exercise protocols may not require the
308 physiological demands of sports with random and multiple changes in speed and direction,
309 future studies should address whether NZBC extract, alone or in combination with other
310 supplements would affect for the performance of field-based sport-specific tests.

311 **Conclusions**

312 Seven days intake of New Zealand blackcurrant extract improved high-intensity
313 intermittent running performance in males, allowed higher lactate values at exhaustion and
314 improved post-exercise recovery. These findings may have implications for nutritional
315 strategies used by athletes involved in sports with repeated sprints.

316

317 Conflict of Interest

318 Supplement (CurraNZ™) for this study was provided by Health Currancy Ltd (United
319 Kingdom). The authors declare no other conflict of interest.

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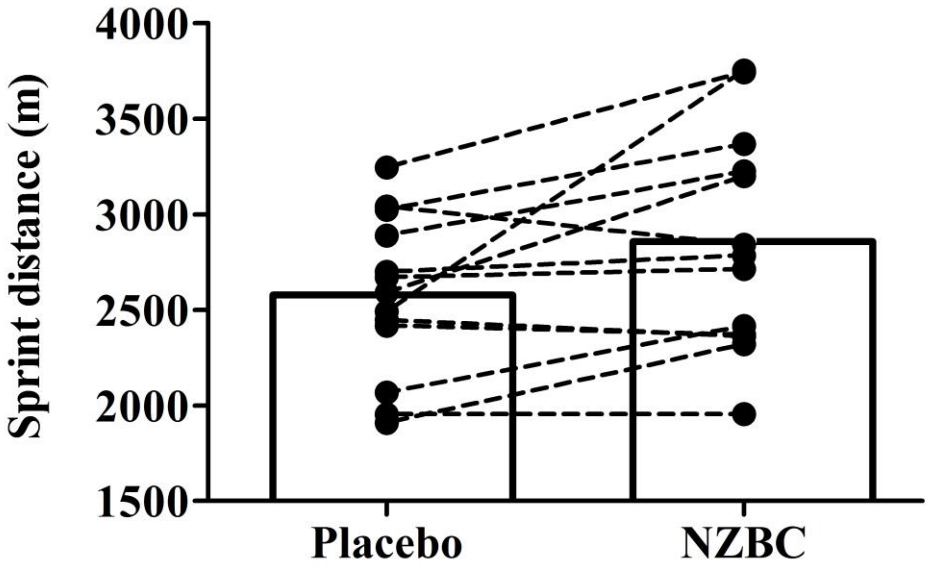
FIGURE LEGENDS



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447 **Figure 1.** Sprint distance during the high-intensity intermittent running protocol. Columns
448 show group means. Symbols and dashed lines show the individual responses. *Sprint distance
449 was increased with NZBC extract (P<0.05).

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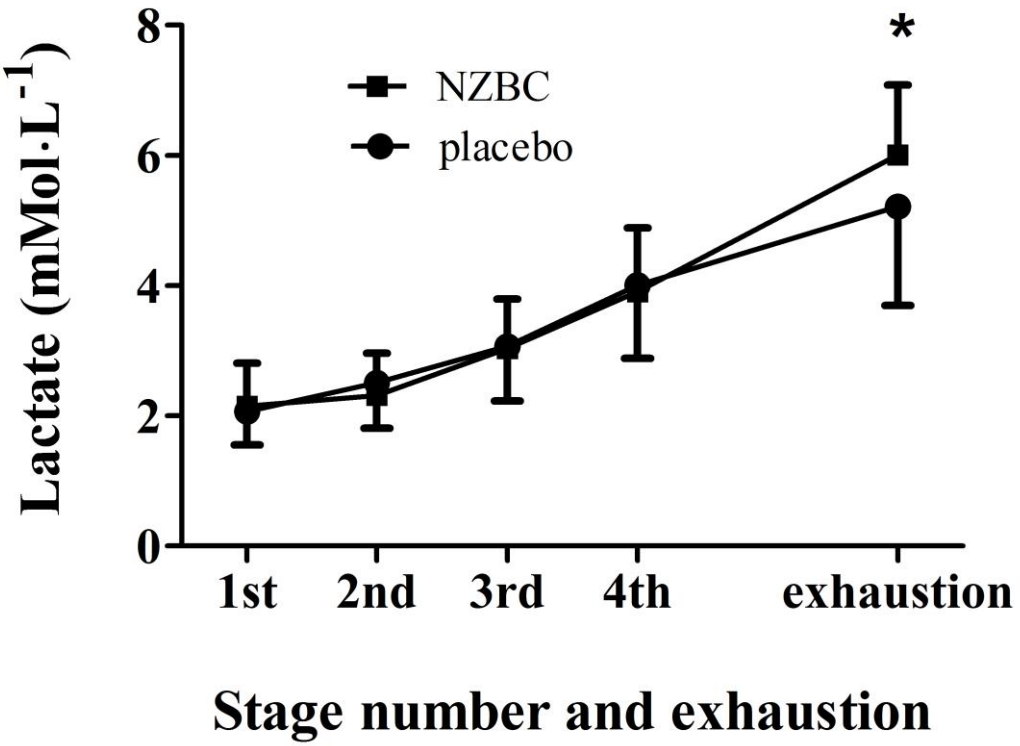

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460 **Figure 2.** Absolute lactate during the first four stages and exhaustion during the high-

461 intensity intermittent running protocol after NZBC extract (squares) and placebo (circles).

462 Data are mean ± SD. * indicates a trend ($0.05>P\leq0.1$).

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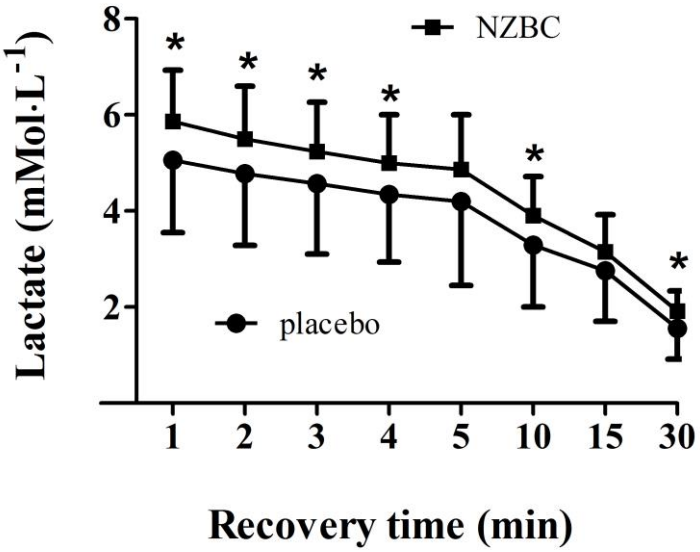

Figure 3. Absolute lactate during 30-minutes of passive recovery following exhaustion by a high-intensity intermittent running protocol after NZBC extract (squares) and placebo (circles). Data are mean ± SD. * indicates a trend ($0.05>P\leq 0.1$).

Table 1. Physiological responses and rating of perceived exertion (RPE) at comparable stages during the high-intensity intermittent running test.

Variable	first	second	third	fourth
Heart rate (beats·min^{-1})				
Placebo	165±10	161±7*	166±8$^{\$}$	171±8*$^{,\$,\#}$
NZBC	165±8	163±8	169±8*$^{,\$}$	173±8*$^{,\$,\#}$
$\dot{V}O_2$ (mL·kg^{-1}·min^{-1})				
Placebo	43.9±3.4	43.2±3.0	45.0±3.2*$^{,\$}$	46.9±3.2*$^{,\$,\#}$
NZBC	45.4±3.5	44.8±3.8	46.3±3.6*$^{,\$}$	48.2±3.7*$^{,\$,\#}$
RPE				
Placebo	12±2	14±2*	16±2*$^{,\$}$	17±2*$^{,\$,\#}$
NZBC	13±2	14±2*	15±2*$^{,\$}$	17±2*$^{,\$,\#}$

Heart rate, lactate and RPE data reported as mean ± SD from 13 participants. $\dot{V}O_2$ data reported as mean ± SD from 9 participants. NZBC, New Zealand blackcurrant. * denotes difference with first stage, $^{\$}$ denotes difference with second stage, # denotes difference with third stage (P<0.05).