Manuscript Title: **The effects of an acute dose of New Zealand blackcurrant extract on 5 km running performance**

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Abstract

This study investigated the effects of an acute dose (900 mg) of New Zealand Blackcurrant (NZBC) extract on 5 km running performance, alongside associated physiological and metabolic responses. Sixteen trained male runners (age 26 ± 5 years, stature 173.4 ± 7.3 cm, body mass, 73.7 ± 6.9 kg, $\dot{V}O_{2max}$ 55.4 ± 6.1 $ml \cdot kg^{-1} \cdot min^{-1}$) ingested either capsules containing NZBC extract (3 x 300 mg CurraNZ™, 315 mg anthocyanins) or a matched placebo (3 x 300 mg gluten free flour) 2 hours before exercise in a double-blind, randomised, crossover design. Performance time, physiological, and metabolic responses were assessed in a 5-km time-trial, preceded by 10 min exercise at the lactate threshold on a treadmill. NZBC extract did not alter the physiological or metabolic responses to exercise at the lactate threshold ($\dot{V}O_2$, RQ, $\dot{V}E$, carbohydrate oxidation, fat oxidation, heart rate, blood lactate or Rating of Perceived Exertion, $P>0.05$). The 5-km time-trial was completed in a faster time in the NZBC extract condition compared to placebo (NZBC: 1308.96 ± 122.36 s, Placebo: 1346.33 ± 124.44, $P=0.001$, $d=-0.23$, CI range=-0.46 to 0.00 s). No differences in physiological or metabolic responses were apparent between conditions for the 5-km time-trial ($P>0.05$). Ingesting 900 mg of NZBC extract as an acute dose improves performance in trained male runners without altering physiological or metabolic responses to exercise. Further research is needed to assess a wider range of possible mechanisms (e.g., cardiovascular function, metabolite profiles) to advance insight into improved performance following supplementation.

Introduction

The use of dietary supplements to enhance performance is commonplace among sports competitors (Knapik et al., 2016). New Zealand Blackcurrant (NZBC) extract contains high concentrations of anthocyanins; delphinidin 3-O-glucoside, delphinidin 3-O-rutinoside, cyanidin 3-O-rutinoside and cyanidin 3-O-glucoside (Cortez & Gonzalez de Mejia, 2019). Anthocyanins are polyphenols from the flavonoid family comprising the red, blue or purple pigments in fruits, flowers and vegetables (Khoo et al., 2017). Dietary anthocyanins have multiple health benefits, including a reduction in cardiovascular disease risk, anti-inflammatory effects, and anticancer activity (Li et al., 2017). Their value in exercise settings has recently been reviewed, revealing anthocyanin-rich NZBC can benefit cycling and running performance (all ≤ 90 min), in addition to physiological and metabolic responses to exercise (Cook & Willems, 2019).

A primary determinant of high-intensity endurance performance is the ability of the cardiovascular system to maintain blood flow to the periphery for oxygen and nutrient delivery, metabolite removal and lactate shuttling to alternative cells (e.g. the liver, heart and kidneys) (Bassett & Howley, 2000). Investigations have revealed that blackcurrant anthocyanin intake confers positive effects on blood flow (Cook et al., 2015; Cook, Myers, Gault, & Willems, 2017; Matsumoto et al., 2005) femoral artery diameter and cardiac output (Cook, Myers, Gault, & Willems, 2017). These alterations are supported by in-vitro studies, which show that anthocyanins upregulate expression of endothelial nitric oxide synthase (eNOS) and subsequent vasorelaxant agent nitric oxide (NO) release (Xu et al., 2004). Although research findings are somewhat equivocal, these alterations in vascular properties could contribute to improved muscle oxygenation (Fryer et al., 2020) and change the balance of lactate appearance and

removal mechanisms to blood lactate accumulation (Willems et al., 2015). Indeed, a rightward and downward shift of the lactate curve at 40, 50, 60 and 70% of maximal cycling power output was observed when NZBC powder (6 g·day^{-1}) was supplemented over a 7-day period (Willems et al., 2015); such changes are often associated with enhanced endurance performance. Several other mechanisms for anthocyanins' effects on endurance are possible. Increases in fat oxidation (17-27%) following 7-days of NZBC extract intake have been reported (Cook et al., 2015; Cook, Myers, et al., 2017b; Strauss et al., 2018), which could promote glycogen sparing (Strauss et al., 2018). Greater NO availability could also favourably affect mitochondrial respiration and sarcoplasmic reticulum Ca^{2+} handling (Jones, 2014), whilst anthocyanins' antioxidant properties (De la Cruz et al., 2013) might limit muscle fatigue associated with reactive oxygen species accumulation (Powers & Jackson, 2008).

While the aforementioned evidence to support a possible endurance performance benefit is promising, studies assessing the effect of NZBC extract on time-trial (TT) performance are minimal, relate principally to cycling, and results are inconsistent. For example, improvements of 2.4% in a 16.1 km cycling TT were reported when trained male cyclists ingested NZBC extract daily (105 mg anthocyanins) for 7 days (Cook et al., 2015). In this study, testing commenced 2 hours after the final dose of NZBC, and so it is not clear if performance was enhanced because of the acute availability of anthocyanins and their metabolites, or some chronic effect of supplementation. However, another study found no performance enhancing effect of NZBC extract during a 7-day supplementation period with cycling TT performance tested on day one, four and seven. Neither, different dosages (105 mg or 210 mg anthocyanin) or the dosing regime (acute or chronic) resulted in faster

114 performance times compared to a placebo. Clearly, further research is required to
115 elucidate whether an acute dose of NZBC extract enhances TT performance in various
116 distances and exercise modalities.
117
118 Therefore, the purpose of this study was to investigate the effects of an acute dose of
119 NZBC extract (900 mg, ~315 mg anthocyanin) on 5 km TT performance in trained
120 male runners. The secondary aim was to assess the physiological and metabolic
121 responses to NZBC during exercise performed at the lactate threshold and during a
122 performance TT.
123
124
125 **Methods**
126
127 *Participants*
128 After obtaining institutional ethical approval and informed consent for the conduct of
129 research in accordance with the Declaration of Helsinki, 16 trained male runners or
130 triathletes (age 26 ± 5 years, stature 173.4 ± 7.3 cm, body mass, 73.7 ± 6.9 kg,
131 $\dot{V}O_{2max}$ 55.4 ± 6.1 $ml \cdot kg^{-1} \cdot min^{-1}$ [range 44.3 – 69.3 $ml \cdot kg^{-1} \cdot min^{-1}$]), volunteered to
132 participate in the study. All participants engaged in a structured training programme
133 and competed in running events regularly. *A priori* sample size calculations based on
134 an effect size of 0.7 (Cook et al., 2015) with α at 0.05 and power at 0.8 identified a
135 necessary sample size of 15. Exclusion criteria were: 5 km personal best time >25
136 min, recent (within 6 months) use of nutrition supplements, and disease history that
137 would impact exercise.
138
139 *Experimental Design*

A repeated-measures, double-blind, randomised (online programme), cross-over study design was employed, with participants attending the laboratory on three separate occasions at the same time of day (<2 hours difference) with all testing taking place in the morning and early afternoon. On the first visit, participants completed a graded incremental treadmill test to volitional exhaustion to establish $\dot{V}O_{2max}$ and running speed at lactate threshold (LT1) (Jones et al., 1999). In an attempt to avoid learning effects, participants were subsequently familiarised with the 5-km running distance, whereby treadmill speed was controlled manually by each participant.

The two subsequent experimental testing visits included two stages; a 10-min run at the individualised speed corresponding to LT1, followed by a 5-km TT. For all visits, the treadmill was set at a 1% gradient to reflect the energetic cost of outdoor running (Jones & Doust, 1996). Measures of oxygen uptake ($\dot{V}O_2$), Respiratory Exchange Ratio (RER), minute ventilation ($\dot{V}E$), carbohydrate oxidation, fat oxidation, heart rate (HR), blood lactate (B[La]) and rating of perceived exertion (RPE) were taken for both stages of the protocol and performance splits and overall time was recorded for the TT. The NZBC extract or placebo was administered in a randomised fashion 2 h prior to each experimental visit. The duration between the first and second visit was at least 48 hours to ensure sufficient recovery, with a washout period >10 days and <16 days between the second and third trials. A 14-day washout period was found to allow biochemical and biomarkers of antioxidant status to return to baseline values after one month of daily (306 mg) anthocyanin (strawberry) intake (Alvarez-Suarez et al., 2014). Participants were instructed to arrive at each visit in a euhydrated state, abstain from strenuous exercise and alcohol 24 hours before, and caffeine on the

day of testing. Aside from these restrictions, maintenance and consistency of their typical weekly exercise schedule over the course of the study was encouraged. To reduce any effect of diet, participants were informed to ingest only water for 2 h prior to experimental testing and food diaries were issued to complete in the 48 h before visit 2. The diaries were handed back to the participants with instructions to replicate in the 48 hours prior to visit 3 and to indicate any deviation of dietary intake that occurred. Adherence to diet replication was confirmed before subsequent testing. During this period, participants were instructed to follow their typical diet, including their typical pre-race meal (>2 h before arrival) for greater ecological validity. Although some studies restrict food-derived anthocyanin sources before exercise testing (Keane et al., 2018), this is thought to limit the ecological validity of nutrition research (Close et al., 2019) as any effect of supplementation could be exaggerated (Morehen et al., 2021). Participants were asked whether they experienced any side effects of supplementation (e.g., gastrointestinal symptoms including nausea, vomiting, stomach pain, bloating, diarrhoea and constipation) at the end of visit 2 and 3. The ambient laboratory conditions (temperature: 21.5 ± 1.3 ºC, humidity: 63 ± 7 %) were controlled to ensure consistency between visits.

Procedures

Graded incremental test

After assessment of anthropometric characteristics, participants completed a graded incremental treadmill test to volitional exhaustion on a motorised treadmill (H/P Cosmos, Pulsar, Nussdorf-Traunstein, Germany) comprising 4-minute stages and increments of 1 $km \cdot h^{-1}$. As the determination of individualised speed at LT1 was required for subsequent visits, starting speed corresponded to 70% of their 10 km

pace, or 80% of marathon pace (Dantas & Doria, 2015); if neither were known then 8 km·h^{-1} was selected. LT1 was defined as the running speed at which blood lactate first rose above resting values (Jones et al., 1999). This exercise intensity was selected due to a) its association with a predictable steady state metabolic response and b) its ability to appropriately account for individual fitness (more so than a % of $\dot{V}O_{2max}$) because it demarcates the transition from the moderate to heavy exercise domain. An online gas analyser (Cosmed Quark RMR, Cosmed S.r.l., Rome, Italy) measured breath by breath $\dot{V}O_2$, RER, and HR (Garmin premium HR, Garmin Ltd, Kansas, USA). Rating of perceived exertion (Borg, 1982) and B[La] (Lactate Pro II, Arkray, Kyoto, Japan) were measured during the last 30 seconds of each stage. Criterion for achievement of $\dot{V}O_{2max}$ was defined as a plateau (<3%) in the oxygen uptake exercise intensity relationship, respiratory exchange ratio ≥1.15, HR within 10 b·min^{-1} of age-predicted maximum, RPE 19-20, BLa >8 mmol^{-1} and volitional exhaustion (Winter, 2006). Regular verbal encouragement was given to ensure maximum effort.

Exercise at the lactate threshold

Upon arrival to the laboratory for the second and third visits, participants assumed a seated position for 5 min before blood pressure of the brachial artery was measured (Omron, M5- I, Omron, Matsusaka Co. Ltd, Japan) to determine systolic, diastolic, and mean arterial pressure (MAP) values, which provided a measure of cardiovascular function at rest. Then, participants completed a 10-minute run at their corresponding LT1 running speed, as previously determined. Heart rate and breath-by-breath analysis were recorded throughout the 10-minute trial to measure effects

214 of NZBC extract on substrate oxidation and gaseous exchange, with measurement
215 of RPE in the final minute and B[La] upon completion.
216
217 *Time-trial performance test*
218 Following 5 min rest, participants completed a self-paced 5 km run as fast as
219 possible from a standing start. Low intra-subject variability of the same distance and
220 protocol has previously been reported (CV 1.5 ± 0.6%, r = 0.99) in competitive male
221 runners (Fisher et al., 2017). Fast acceleration to the desired running speed was
222 achieved via a built-in treadmill function, which participants controlled, having been
223 familiarised with the process in their first laboratory visit. Thereafter, participants
224 manually altered their own treadmill speed using up and down arrows but all metrics
225 apart from the cumulative distance were occluded to prevent fixed pacing. Time to
226 complete the 5-km TT, split times of each km, and $\dot{V}O_2$, RER, $\dot{V}E$, fat oxidation,
227 carbohydrate oxidation, and HR were measured continuously. RPE, B[La] and
228 average running speed were measured on completion of each kilometre. Participants
229 then returned to the laboratory for the third and final visit where they received either
230 placebo or NZBC extract. All procedures as completed during the second visit were
231 then repeated.
232
233
234 *Supplemental protocol*
235 The supplements were distributed at the end of visit 1 and visit 2 in a randomised
236 and double-blind fashion. A study by Hurst et al. (Hurst et al., 2019) reported peak
237 plasma anthocyanin concentration occurred after 2 hours of ingestion of NZBC
238 extract, therefore in accordance with these findings athletes were instructed to ingest
239 the supplement, 2 h prior to experimental trials, which was confirmed upon arrival.

240 The three NZBC (3 x 300 mg active cassis containing a total of 315 mg
241 anthocyanins) and placebo (3 x 300 mg gluten-free flour) capsules were opaque and
242 hence were not distinguishable by the researcher or participant. The ingredients for
243 NZBC extract (315 mg anthocyanins, 35–50 % delphinidin- 3-rutinoside, 5–20 %
244 delphinidin-3-glucoside, 30–45 % cyanidin-3-rutinoside, 3–10 % cyanidin-3-
245 glucoside; CurraNZTM, Health Currancy Ltd., Surrey, UK) have previously been
246 reported by Cook et al. (2015).
247
248 **Statistical analysis**
249 Data was analysed using SPSS v26 (SPSS Inc., Chicago, IL, USA). Data normality
250 was assessed using Shapiro-Wilk test. Homogeneity was tested using Mulchey's
251 Test of Sphericity, with Greenhouse-Geiser adjustments used for violations ($P<0.05$).
252 Paired samples *t*-tests were used to compare responses between NZBC extract and
253 placebo conditions for the 10-min exercise at LT. A 2-way repeated measures
254 analysis of variance (RM-ANOVA) (condition [2], distance [5]) was used to compare
255 differences between NZBC extract and placebo over each km for all variables, with
256 completion of subsequent post-hoc tests (Bonferroni) for significant effects. Analysis
257 was supplemented by calculation of effect sizes (Cohens *d*) with accompanying 95%
258 confidence intervals, considered as small (0.2-0.59), moderate (0.6-1.19), large (1.2-
259 2.0) and very large (>2.0) (Hopkins et al., 2009). The following equations were used
260 to calculate rates of whole-body fat and carbohydrate oxidation ($g \cdot min^{-1}$) during
261 exercise: Carbohydrate oxidation = (4.55 × VCO2) – (3.21 × VO2), Fat oxidation =
262 (1.67 × VO2) – (1.67 × VCO2). Equations were modified to account for nitrogen
263 production as recommended (Frayn, 1983). In instances where the RER value ≥1, a

value of 0 was inserted for fat oxidation analysis. Data is expressed as mean ± standard deviation whilst significance was set at alpha level $P \leq 0.05$.

Results

Mean arterial pressure

Mean arterial pressure after supplementation was not different between conditions (NZBC: 94 ± 4 mmHg, Placebo: 93 ± 4 mmHg, P= 0.33). Systolic and diastolic pressures were 125 ± 6 / 77 ± 5 mmHg for the placebo and 125 ± 5 / 79 ± 5 mmHg for the NZBC condition, respectively.

Exercise at the lactate threshold

During the run at LT1, there were no significant differences between NZBC and placebo for $\dot{V}O_2$, RER, $\dot{V}E$, carbohydrate oxidation, fat oxidation, HR, B[La] or RPE, (*P>0.05*; Table 1).

***** Insert Table 1 here *****

5-km running Time-Trial

Completion time and running speed

The NZBC extract condition reduced the time taken to complete the 5-km TT compared to placebo (P = 0.001; NZBC: 1308.96 ± 122.36 s, Placebo: 1346.33 ± 124.44 s, d=-0.23, CI range= -0.38 to -0.08) with a group mean reduction of 2.9 ± 2.97 % (range -0.9 to 5.7 %) and 14 of the 16 participants improving their performance time (Fig 1A). Although there were no significant interaction effects, small effect sizes favoured NZBC extract, evidenced by faster times at km 1 (271 ± 25 *cf.* 280 ± 28 s; d=-0.25, CI range=-0.47 to -0.02), 2 (258 ± 27 *cf.* 269 ± 28 s; d=-

293 0.30, CI range= -0.59 to -0.01) and 4 (265 ± 29 *cf.* 273 ± 27 s; *d*=0.23, CI range=-
294 0.46 to 0.00). Time taken to complete each km alongside running speed can be
295 found in Fig 2a/b.
296
297 ***** Insert Figure 1A and 1B here *****
298
299 ***** Insert Figure 2A and 2B here *****
300
301 *Physiological and metabolic variables*
302 No significant main effects for condition or interaction effects were observed for $\dot{V}O_2$
303 ($ml \cdot kg^{-1} \cdot min^{-1}$), RER, VE, HR, B[La], RPE carbohydrate oxidation or fat oxidation
304 (*P*>0.05). However, significant effects for distance were found for all variables (all
305 *P*<0.001 to P=0.017). All data can be found in Table 2.
306 **Discussion**
307
308 The principal finding of the current study is that an acute dose (315 mg anthocyanin)
309 of NZBC extract resulted in faster completion (38 s, ~3%) of a 5-km treadmill running
310 TT when compared to a placebo. Faster running speeds in NZBC were produced
311 without significant changes in the physiological, metabolic and perceptual responses
312 to the TT. There were also no significant differences in responses to exercise at the
313 lactate threshold between conditions. This study is the first to investigate and
314 advocate an acute dose of NZBC extract taken 2 h before exercise to improve 5 km
315 endurance performance in well-trained runners.
316
317 The 3% (38 s, *d* = 0.23) improvement in 5-km running performance after ingestion of
318 NZBC extract is likely to be meaningful, given the typical variation in 5 km running

time in trained runners (1.5%/14.25 s) and the magnitude of the observed effect size (Fisher et al., 2017). Assessment of individual responses revealed that eleven participants improved by more than 14.25 s (range 34 – 126 s). Percentage improvements were similar to most previous TT studies (1.0 - 2.4%) (Braakhuis et al., 2014; Cook et al., 2015; Murphy et al., 2017) and are consistent with the small significant effect size (0.45) shown in a meta-analysis of NZBC on time-to-fatigue and TT performance tests (Braakhuis et al., 2020). Importantly though, all previous studies reporting performance improvements used a loading phase of daily blackcurrant anthocyanin supplementation (typical dose: 105 – 210 mg) for at least 7 days prior to exercise. In the two studies to test the effects of an acute dose (105 and 210 mg anthocyanin, (Montanari et al., 2020), and 300 mg (Montanari et al., 2023)), no benefit on 16.1 km cycling time-trial performance was reported. Interestingly though, upon completion of a sub-group analysis, the Montanari et al. (2023) study found that slower cyclists (<1400 s) performed quicker in the NZBC extract condition with a comparable effect size to that of the present study (*d*=-0.23). Therefore, notwithstanding the potential role that training status might play, these studies suggest that performance enhancement after ingesting a single dose of NZBC extract (3 capsules; 315 mg anthocyanins taken 2 h pre-exercise) is comparable to taking one or two daily capsules for a week up until the day of performance. These findings are valuable to athletes and exercisers, making it possible to confer benefits via a more practical and cost-effective supplementation strategy, without any reported negative side-effects from the prescribed dose.

The faster performance times in the TT were accomplished without participants

experiencing elevated physiological and metabolic responses. The exercise at the lactate threshold, undertaken in an attempt to better understand the possible mechanisms for performance changes, revealed a small reduction in B[La] with NZBC (*d*=-0.22), however the accompanying confidence intervals are compatible with the effect being substantially positive (*d*=0.21) and negative (-0.65). Reductions in B[La] in endurance-trained males supplementing with NZBC have previously been reported (Willems et al., 2015) although another study reported no change (Montanari et al., 2020). Willems et al. (2015) found that NZBC powder (138.6 mg daily anthocyanins for 7 days) mediated a downward and rightward lactate shift (13-27% lower) during a steady-state non-continuous incremental exercise test (40-70% of maximal power output), suggesting that supplementation might influence lactate appearance and removal mechanisms. Acute dosing of blackcurrant anthocyanin can increase peripheral blood flow (Matsumoto et al., 2005) and vasodilation via activation of endothelial nitric oxide synthase and subsequent nitric oxide production in-vitro (Ziberna et al., 2013). Therefore, improved performance outcomes might be explained by arterial dilation and increased blood flow to the working muscle, which is also associated with greater lactate clearance (Willems et al., 2015). Further work should assess the role of NZBC extract specific anthocyanin metabolite profiles, as metabolites in similar compounds (e.g. Montmorency tart cherry) have been identified as possible drivers of change in vasculature (Keane et al., 2016).

We observed no further differences in the physiological, metabolic and perceptual responses to the exercise at lactate threshold and 5 km TT with acute NZBC extract supplementation, therefore the precise mechanisms for improved performance are unclear. Whilst the faster TT performances in NZBC occurring for similar

368 physiological responses could be interpreted as a change in the relationship
369 between exercise intensity and physiological response, we are cautious about
370 interpreting non-significant effects as being statistically equivalent.
371
372 Changes in cardiovascular function with chronic NZBC supplementation have been
373 reported; specifically, increases in cardiac output at rest (Cook, Myers, et al., 2017a;
374 Willems et al., 2015) and during submaximal isometric contractions (Cook, Myers,
375 Gault, & Willems, 2017), as well as increases in peripheral blood flow (Matsumoto et
376 al., 2005). However, these findings are not consistent (Willems et al., 2015). Cardiac
377 output and peripheral blood flow were not measured in our study, and it is possible
378 that they contributed to changes in performance; however, we observed similar heart
379 rates between conditions (178 *cf.* 179 $b \cdot min^{-1}$) in the TT and during exercise at the
380 lactate threshold (152 *cf.* 156 $b \cdot min^{-1}$), and so this aspect of cardiovascular function
381 is unlikely to be affected by acute NZBC supplementation. The extent to which
382 cardiovascular changes occur appear to be dose-dependent (Cook, Myers, et al.,
383 2017b). Future studies should therefore examine the effect of a similar acute dose of
384 NZBC extract (315 mg anthocyanins) on cardiovascular function.
385
386 Improved muscle oxygenation was recently reported during isometric (Fryer et al.,
387 2020) and submaximal intermittent exercise (Cook, Myers, et al., 2017b) after a 7-
388 day intake of NZBC extract (210 mg anthocyanins). Increased oxygen delivery could
389 increase the contribution of aerobic metabolism and subsequently result in reduced
390 production of blood lactate and associated metabolites (Bassett & Howley, 2000).
391 However, other studies have failed to show that NZBC influences the oxygen cost of
392 exercise (138.6 mg daily anthocyanins for 7 days; (Willems et al., 2015), which

agrees with our data during exercise at the lactate threshold and the TT. This suggests that enhanced TT performance with acute NZBC supplementation is not due to a NO-mediated change in oxygen consumption during exercise.

Substrate oxidation remained similar between conditions during exercise at the lactate threshold (NZBC: 0.40 g·min^{-1}; PLA: 0.39 g·min^{-1}). Our results do not support the augmentation in whole-body fat oxidation during exercise previously reported in trained cyclists after a 7-day daily dosing period (105 mg anthocyanins, 10-120 min, 45-65% VO2 max) (Cook et al., 2015; Cook, Myers, et al., 2017b; Strauss et al., 2018) or when a comparative dose (to the current study; 315 mg anthocyanins) was used for 7 days (Cook, Myers, et al., 2017b). Our fat oxidation values are similar to those studies delivering lower dosages acutely 2 h prior to exercise (300 g 0.34 g·min^{-1}; 600 g 0.40 g·min^{-1}, (Montanari et al., 2020). Collectively, results suggest that the mechanisms responsible for initiating lipolysis might be dependent on cumulative, or more consistent (e.g., daily) delivery of NZBC. The suggested mechanism that NZBC anthocyanins or their metabolites influence the key proteins regulating lipolysis (Strauss et al., 2018) requires further study.

This study has some limitations. Firstly, it did not control for or analyse anthocyanin intake from meals prior to exercise. Although this ensured that participants consumed and replicated a pre-race meal that was ecologically valid for all visits, knowledge of prior anthocyanin ingestion would have been useful to determine the extent to which the anthocyanin content of foods could have influenced the outcome. However, previous studies show average dietary anthocyanin intake to be low (e.g., 12.5 mg/day in the US (Wu et al., 2006), and 19.8 - 64.9 mg/day and 17.7 – 44.1

mg/day in European males and females (Zamora-Ros et al., 2011), respectively) in relation to the larger dose (~315 mg) provided in the current study. Secondly, our study did not assess all plausible mechanisms that could account for performance differences with administration of the selected dose, which should be assessed in future studies.

In conclusion, this is the first study to demonstrate meaningful improvements in 5 km running performance with ingestion of a single dose (3 capsules; 315 mg anthocyanins) of NZBC extract. Assessment of a wider range of mechanisms (e.g., cardiovascular function measures, metabolite profiles) could advance insight into the enhanced TT performance with an acute dose.

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597 **Table 1**. Physiological and metabolic responses to 10-minute exercise at the lactate threshold.
598 Data are mean ± SD with effect sizes ± 95% confidence intervals.
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Variable	Placebo	NZBC	*d*	CI (range)
$\dot{V}O_2$ (L·min^{-1})	3.08 ± 0.26	3.03 ± 0.32	-0.14	(-0.38 - 0.11)
$\dot{V}O_2$ (ml·kg^{-1}·min^{-1})	41.98 ± 4.61	41.24 ± 4.43	-0.12	(-0.32 - 0.08)
$\dot{V}O_2$ (% of max)	76.01 ± 5.92	74.79 ± 7.11	-0.16	(-0.43 – 0.12)
$\dot{V}CO_2$ (L·min^{-1})	2.86 ± 0.31	2.79 ± 0.28	-0.16	(-0.45 – 0.13)
RER	0.93 ± 0.06	0.92 ± 0.05	0.09	(-0.60 – 0.41)
$\dot{V}E$ (L·min^{-1})	83.13 ± 12.71	83.38 ± 11.50	0.02	(-0.10 – 0.13)
CH_{ox} (g·min^{-1})	3.12 ± 0.89	2.98 ± 0.56	-0.12	(-0.55 –0.31)
FAT_{ox} (g·min^{-1})	0.39 ± 0.27	0.40 ± 0.20	0.04	(-0.41 – 0.50)
Heart rate (b·min^{-1})	152 ± 15	156 ± 14	0.17	(0.00 – 0.35)
Lactate (mmol·L^{-1})	2.88 ± 1.86	2.33 ± 1.11	-0.22	(-0.65 – 0.20)
RPE (AU)	11.6 ± 1.41	11.4 ± 1.45	-0.10	(-0.43 – 0.23)

600 Note: CH_{ox} = Carbohydrate oxidation; FAT_{ox} = Fat oxidation

Table 2 Physiological and metabolic data recorded during the 5 km running time-trial. Data are mean ± SD.

Condition	1 km	2 km	3 km	4 km	5 km	Overall	*d* and CI (range)
$\dot{V}O_2$ ($ml \cdot kg^{-1} \cdot min^{-1}$)							
NZBC	43.66 ± 4.16	50.47 ± 4.76	50.85 ± 5.14	51.20 ± 5.55	52.24 ± 5.54	49.68 ± 4.87	0.05 (-0.15 – 0.25)
Placebo	44.55 ± 4.15	49.81 ± 5.19	50.42 ± 5.44	50.45 ± 5.29	51.54 ± 5.39	49.35 ± 4.91	
$\dot{V}O_2$ ($L \cdot min^{-1}$)							
NZBC	3.20 ± 0.30	3.70 ± 0.28	3.72 ± 0.27	3.75 ± 0.26	3.82 ± 0.26	3.64 ± 0.26	0.07 (-0.21 – 0.35)
Placebo	3.27 ± 0.27	3.65 ± 0.30	3.69 ± 0. 30	3. 69 ± 0.27	3.77 ± 0.27	3.61 ± 0.26	
$\dot{V}CO_2$ ($L \cdot min^{-1}$)							
NZBC	3.04 ± 0.35	3.65 ± 0.36	3.63 ± 0.32	3.62 ± 0.31	3.74 ± 0.33	3.53 ± 0.30	0.17 (-0.16 – 0.49)
Placebo	3.07 ± 0.37	3.56 ± 0.36	3.56 ± 0.30	3.53 ± 0.25	3.64 ± 0.27	3.47 ± 0.28	
RER (AU)							
NZBC	0.95 ± 0.08	0.99 ± 0.06	0.98 ± 0.06	0.97 ± 0.05	0.98 ± 0.06	0.97 ± 0.06	0.15 (-0.40 – 0.69)
Placebo	0.94 ± 0.07	0.98 ± 0.05	0.97 ± 0.05	0.96 ± 0.05	0.97 ±0.05	0.96 ± 0.05	
$\dot{V}E$ ($L \cdot min^{-1}$)							
NZBC	95.31 ± 12.78	119.12 ± 18.63	125.31 ± 19.91	129.43 ± 19.28	138.17 ± 18.12	121.46 ± 17.06	0.23 (0.10 – 0.59)
Placebo	94.02 ± 13.84	113.80 ± 15.24	119.81 ± 16.21	124.10 ± 17.64	132.57 ± 16.69	116.86 ± 15.14	
Heart rate ($b \cdot min^{-1}$)							
NZBC	164 ± 12	178 ± 11	181 ± 11	184 ± 10	188 ± 10	179 ± 10	0.09 (-0.08 – 0.30)
Placebo	164 ± 13	177 ± 12	181 ± 11	182 ± 11	185 ± 11	178 ± 11	
Blood Lactate ($mmol \cdot L^{-1}$)							
NZBC	6.54 ± 3.0	8.61 ± 4.85	9.16 ± 4.75	9.88 ± 3.11	12.21 ± 3.34	9.28 ± 3.15	0.15 (-0.08 – 0.38)
Placebo	5.62 ± 3.55	7.52 ± 5.17	10.02 ± 6.52	8.96 ± 3.59	10.48 ± 3.57	8.52 ± 3.86	

RPE (AU)							
NZBC	13 ± 1	15 ± 1	16 ± 1	17 ± 1	19 ± 1	16 ± 2	0.18 (-0.35 – 0.26)
Placebo	13 ± 2	14 ± 1	15 ± 2	17 ± 2	19 ± 2	16 ± 2	
Carbohydrate oxidation ($g \cdot min^{-1}$)							
NZBC	3.56 ± 1.03	4.72 ± 1.08	4.57 ± 0.95	4.45 ± 0.90	4.73 ± 1.03	4.40 ± 0.90	0.19 (-0.31 – 0.69)
Placebo	3.49 ± 1.10	4.47 ± 0.97	4.36 ± 0.81	4.23 ± 0.75	4.43 ± 0.86	4.19 ± 0.82	
Fat oxidation ($g \cdot min^{-1}$)							
NZBC	0.32 ± 0.24	0.18 ± 0.23	0.22 ± 0.21	0.26 ± 0.23	0.23 ± 0.22	0.24 ± 0.21	-0.10 (-0.57 – 0.37)
Placebo	0.36 ± 0.30	0.22 ± 0.25	0.24 ± 0.27	0.29 ± 0.27	0.26 ± 0.29	0.27 ± 0.26	

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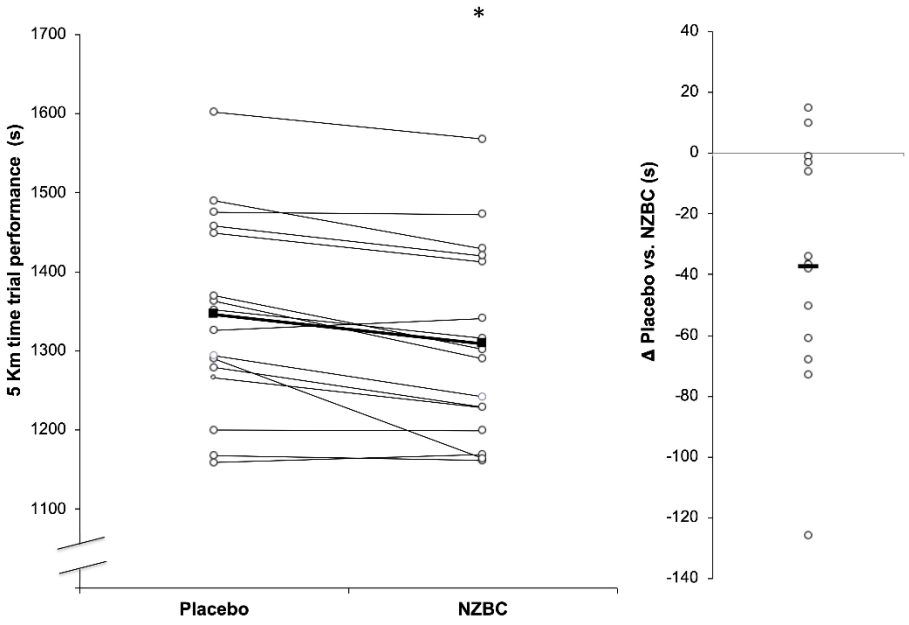

Figure 1A. Overall time during a 5-km running time-trial. Bold black line and squares show group mean ± SD. Individual responses are shown with lighter black lines. * indicates significantly faster 5-km time-trial in NZBC (*P*=0.001). **1B.** shows individual differences (circles) in seconds between placebo and NZBC.

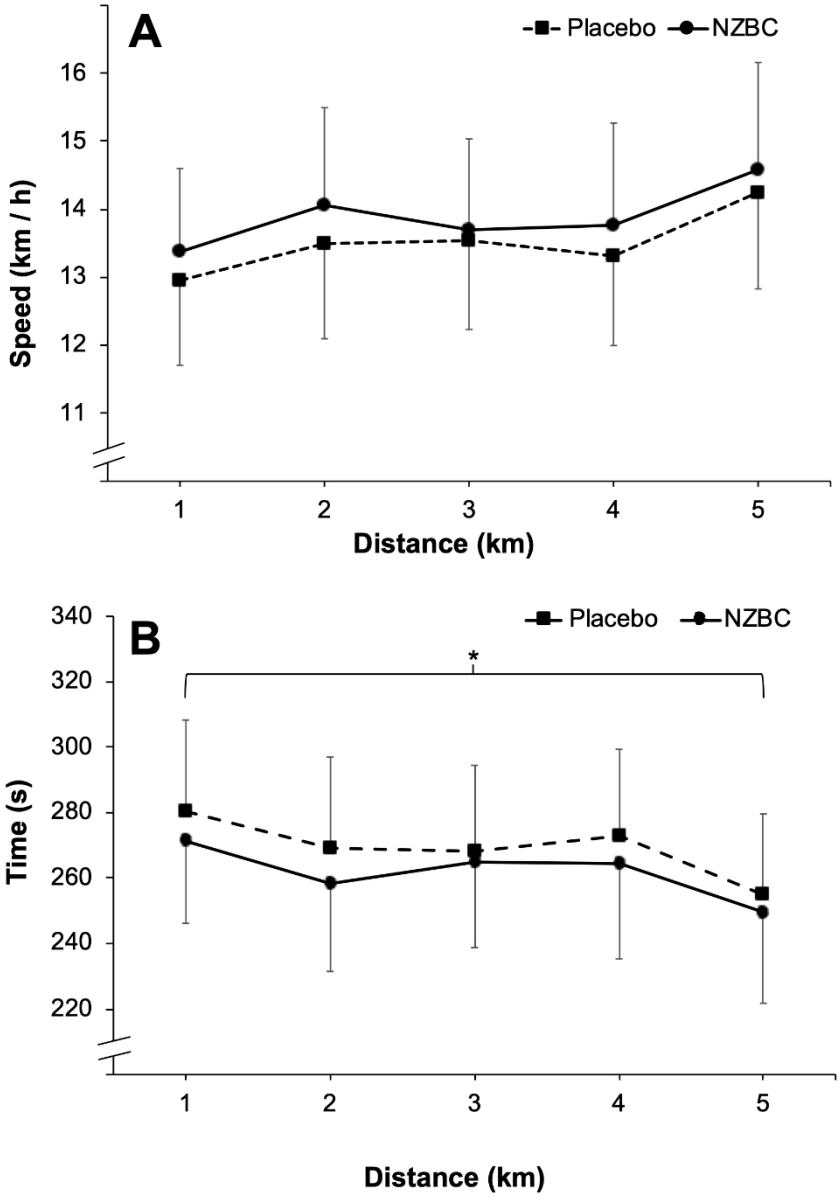

Figure 2A. Running speed (km / h) and **2B.** completion time per km (s) throughout the 5 km time-trial.

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