

1 Title of Article: New Zealand Blackcurrant Extract Improves Cycling Performance and Fat
2 Oxidation in Cyclists
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29 **ABSTRACT**

30 **PURPOSE:** Blackcurrant intake increases peripheral blood flow in humans, potentially by anthocyanin-induced
31 vasodilation which may affect substrate delivery and exercise performance. We examined the effects of New
32 Zealand blackcurrant (NZBC) extract on substrate oxidation, cycling time-trial performance and plasma lactate
33 responses following the time-trial in trained cyclists.

34 **METHODS:** Using a randomized, double-blind, crossover design, fourteen healthy men (age: 38 ± 13 years, height:
35 178 ± 4 cm, body mass: 77 ± 9 kg, $\dot{V}O_{2\max}$: 53 ± 6 ml·kg⁻¹·min⁻¹, mean \pm SD) ingested NZBC extract (300 mg·day⁻¹
36 CurraNZ™ containing 105 mg anthocyanin) or placebo (PL, 300 mg microcrystalline cellulose M102) for 7-days
37 (washout 14-days). On day 7, participants performed 30 min of cycling (3x10 min at 45, 55 and 65% $\dot{V}O_{2\max}$),
38 followed by a 16.1 km time-trial with lactate sampling during a 20-minute passive recovery.

39 **RESULTS:** NZBC extract increased fat oxidation at 65% $\dot{V}O_{2\max}$ by 27% ($P < 0.05$) and improved 16.1 km time-
40 trial performance by 2.4% (NZBC: 1678 ± 108 s, PL: 1722 ± 131 s, $P < 0.05$). Plasma lactate was higher with NZBC
41 extract immediately following the time-trial (NZBC: 7.06 ± 1.73 mmol·L⁻¹, PL: 5.92 ± 1.58 mmol·L⁻¹ $P < 0.01$).

42 **CONCLUSIONS:** Seven days intake of New Zealand blackcurrant extract improves 16.1 km cycling time-trial
43 performance and increases fat oxidation during moderate intensity cycling.

44

45 **Keywords** time-trial; substrate oxidation; lactate; recovery; anthocyanin; indirect calorimetry; New Zealand
46 blackcurrant; sports nutrition

47

48 Abbreviations:

49	CHox	Carbohydrate oxidation
50	FATox	Fat oxidation
51	NZBC	New Zealand Blackcurrant
52	PL	Placebo
53	$\dot{V}O_{2\max}$	Maximal oxygen uptake
54	WR _{max}	Maximum work rate

55

56

57 **INTRODUCTION**

58 Blackcurrant (*Ribes nigrum*) is a food source rich in polyphenols, including the anthocyanins delphinidin-3-
59 rutinoid, delphinidin-3-glucoside, cyanidin-3-rutinoid and cyanidin-3-glucoside, in addition to some flavanols
60 and flavonols in smaller quantities. Anthocyanins are a flavonoid group that has been associated with benefits for
61 human health through anti-inflammatory effects (Zhu et al. 2013) and anti-oxidant activity (De la Cruz et al. 2013).
62 These effects are of interest to counteract the production of reactive oxygen species during exhaustive exercise (Viña
63 et al. 2000), which is the primary cause of exercise-induced disturbance in the oxidation-reduction status (i.e. redox
64 balance) of skeletal muscle (Powers et al. 2004). In addition, blackcurrant intake has also been reported to increase
65 peripheral blood flow by 22% during typing work in humans (Matsumoto et al. 2005), and retina blood flow in
66 patients with normal tension glaucoma (Ohguro et al. 2007) potentially by anthocyanin-induced vasorelaxation and
67 vasodilation as shown in thoracic aortic rings in male Wistar rats (Zibera et al. 2013). This may be mediated by the
68 ability of anthocyanins to increase nitric oxide by endothelial cells and also a reduced breakdown of nitric oxide by
69 free radicals (Martin et al. 2002; Nagi et al. 2002).

70 The evidence that blackcurrant can improve blood flow and reduce oxidative stress may represent a
71 potential ergogenic affect upon exercise performance in an event with a large aerobic component such as a 16.1 km
72 time-trial as restricted blood flow is considered an important limiting factor in muscle oxygenation during high
73 intensity exercise (Basset and Howley 2000). However, the effects of short duration (7-days) blackcurrant intake on
74 endurance exercise performance have not been examined. Following blackcurrant supplementation (300 mg·day⁻¹
75 anthocyanin) alongside a short-duration (i.e. 3 weeks) high-intensity training programme in 23 female runners,
76 Braakhuis et al. (2014) reported a possible peak running speed improvement of $1.9 \pm 2.5\%$ during an incremental
77 running test of the fastest runners (i.e. runners faster by 1 SD of mean speed on an incremental running test) in the
78 study cohort. However, Skarpańska-Stejnborn et al. (2006) reported no change in best effort 2000m rowing
79 ergometer performance in rowers taking blackcurrant (250 mg blackcurrant powder, 3 times daily) for 6 weeks in a
80 training camp. Both of these studies supplemented athletes over a training period with physiological assessment
81 before and after training with different daily doses and supplementation periods. The dose- and time-dependent
82 responses of blackcurrant on physiological responses are unknown. In addition, no studies have addressed the
83 potential ergogenic properties of short-term (7 days) blackcurrant supplementation on a performance-based test that
84 simulates competition in a trained population without a training period. As anthocyanins reach maximum serum

85 concentrations in 1.81 ± 0.16 h following ingestion, and metabolites remain in the blood stream for at least 48 hours
86 (Czank et al. 2013), a potential build-up of metabolites from a short-term intake (i.e. 7-days) and the subsequent
87 physiological responses such as, altered nitric oxide availability and increased peripheral blood flow, may alter
88 exercise performance. **It should be noted however, that the acute and chronic responses of anthocyanin intake on**
89 **exercise performance are not known, but the chronic exposure as used in the above training studies may result in**
90 **different physiological responses during the training period which may alter the training adaptations, than the**
91 **physiological responses that result from 7-days exposure which may improve performance in high intensity exercise**
92 **with a large aerobic component.**

93 As lactate redistribution following exercise occurs via blood flow (Gladden 2004), an improved peripheral
94 blood flow induced by anthocyanin related vasodilation may benefit lactate removal through greater uptake by liver,
95 heart, kidney and skeletal muscles. Nutritional interventions that improve blood lactate responses after high intensity
96 exercise are therefore of interest to athletes to promote faster recovery, slow lactate accumulation and potentially
97 influence the performance of subsequent high intensity exercise.

98 Experimental studies have also indicated that consumption of some of the anthocyanins within blackcurrant
99 in C57BL/6 mice can inhibit body mass gain, positively alter insulin responses, attenuate lipid accumulation and
100 decrease leptin secretion (Benn et al. 2014) and also enhance adipokine secretions in rat adipocytes (Tsuda et al.
101 2004). These physiological responses may alter fat oxidation during low and moderate intensity exercise where fat
102 oxidation rates are highest (Achten et al. 2002).

103 Therefore, the objectives of the present study addressed whether there are effects of short-term (7-days)
104 NZBC extract on performance, metabolic and physiological responses. The first objective was to examine the effect
105 of New Zealand blackcurrant (NZBC) extract on substrate oxidation **at three different exercise intensities.** The
106 second objective was to examine the effects of NZBC extract on 16.1 km (10-mile) cycling time-trial performance.
107 The third objective was to examine the **lactate responses** following the 16.1 km time-trial. It was hypothesized that
108 NZBC extract would enhance endurance performance, increase fat oxidation and **alter lactate responses during**
109 **passive post-exercise recovery.**

110

111 **METHODS**

112

113 **Participants**

114 Fourteen healthy men volunteered and provided written informed consent to participate in the study with participant
115 characteristics presented in Table 1. Participants were recruited from local cycling and triathlon clubs with a history
116 of sport participation of greater than 3 years and were not involved in a structured training programme at the time of
117 the study, but typically performed cycling exercise of 8 to 10 hours a week. All participants had a personal best time
118 for a 16.1 km cycling time-trial of less than 30 minutes. Participants were screened for intake of other dietary
119 supplements before commencing participation with only one participant required to undergo a wash out period of 14
120 days for taking beetroot supplements. The study was approved by the University of Chichester Research Ethics
121 Committee with protocols and procedures conformed to the 2013 Declaration of Helsinki. Participants did not
122 receive payment for participation.

123 **Experimental Design**

124 Each participant visited the laboratory for 4 morning sessions (<2 hours difference). In preparation for all testing
125 sessions, participants were instructed to abstain from strenuous exercise for 48 hours prior, alcohol intake for 24
126 hours prior and caffeine-containing products on the day of testing. All exercise was performed with the participant's
127 own cycling shoes and pedals attached to the SRM ergometer (SRM ergometer, SRM International, Germany).
128 Saddle height and setback, handle bar reach and drop were personalized in the first visit and replicated for all
129 additional visits. On the first visit, participants stature (Seca 213, Seca, Birmingham, UK), body mass (Kern ITB,
130 Kern, Germany) and body fat (Tanita BC418 Segmental Body Composition analyzer, Tanita, Illinois, USA) were
131 measured. Subsequently, participants completed an intermittent incremental-intensity cycling test until a blood
132 plasma lactate $\geq 4 \text{ mmol}\cdot\text{L}^{-1}$ was obtained. This was followed by a familiarization of the 16.1 km time-trial. In the
133 second visit, participants completed a maximal incremental cycling test to volitional exhaustion to allow
134 measurement of maximal oxygen uptake ($\dot{V}\text{O}_{2\text{max}}$) and maximum work rate (WR_{max} ; the last completed work rate,
135 plus the fraction of time spent in the final non-completed work rate multiplied by the work rate), followed by a rest
136 period and a second 16.1 km time-trial for familiarization.
137 Prior to visits 3 and 4, participants consumed 1 capsule of concentrated NZBC extract (300 mg active cassis
138 containing 105mg of anthocyanins, i.e. 35-50% delphinidin-3-rutinoside, 5-20% delphinidin-3-glucoside, 30-45%
139 cyanidin-3-rutinoside, 3-10% cyanidin-3-glucoside) (CurraNZTM, Health Currancy Ltd, Surrey, UK) or an identical
140 looking placebo capsule (300mg microcrystalline cellulose M102) every morning with breakfast for 7 days. The

141 NZBC capsules were independently analysed for contents, which confirmed ingredients present and that ingredients
142 such as caffeine were absent. On the morning of the final day of supplementation, participants reported to the
143 laboratory at the same time of day, approximately 2 hours postprandial of a standard breakfast (i.e. one slice of
144 buttered toast or bread) and their last supplement capsule. On arrival, participants rested for 10 minutes before their
145 blood pressure was taken four times using an automated cuff (OMRON 705 IT, Medisave, Weymouth, UK) with the
146 last three measurements averaged for quantification of blood pressure. Subsequently, a finger prick blood sample
147 was taken to record resting blood plasma lactate and glucose (YSI 2300 Stat Plus, Yellow Springs Instruments Co.
148 Inc., Yellow Springs, USA). After the resting sample was provided, participants performed a continuous 30 min
149 cycling protocol, consisting of three 10 min stages at 45, 55 and 65% $\dot{V}O_{2max}$ with expired gas samples collected and
150 analysed. Following a 15-minute rest, participants performed a 16.1 km best effort time-trial on the SRM ergometer.
151 The two experimental conditions (NZBC and placebo) were performed in a randomized, double-blind, cross-over
152 design with a 14-day washout period. **An anthocyanin intake three times higher than our study for one month**
153 **reported return to baseline of biochemical parameters and biomarkers of antioxidant status after 15 days washout**
154 **(Alvarez-Suarez et al. 2014)**. Six participants received NZBC extract as first condition. All exercise tests were
155 conducted in a temperature-controlled laboratory at 18°C.

156 **Physical Activity and Dietary Standardization**

157 Participants were instructed to keep their weekly exercise schedule as consistent as possible. Each participant
158 recorded their dietary intake on a written food diary for the 48 hours prior to the first of the experimental condition
159 visits (visit 3). Participants were instructed to replicate this diet for the 48 hours prior to the second experimental
160 condition visit (visit 4) using their previous food diary as a guide, while recording on a new diary their dietary intake
161 for that visit. Food diaries were analysed using Nutritics (Nutritics LTD, Dublin, Ireland) for carbohydrate, fat and
162 protein intake and total energy intake (kJ). There were no differences in absolute or relative to per kilogram of body
163 mass for carbohydrate, fat protein and total energy intake ($P > 0.05$) between the experimental visits (Table 2).

164 Analysis of diaries demonstrated a 100% reported adherence to dietary instructions. Participants reported 100%
165 compliance to the supplementation protocol.

166 **Incremental cycling test**

167 The intermittent incremental cycling test in visit 1 was performed to establish the relationship between oxygen
168 uptake and submaximal power outputs. The protocol began at 50 W for 4 minutes with subsequent stages increasing

169 by 30 W every 4 minutes. Between each exercise stage, participants rested on the ergometer without pedalling for 2
170 minutes, in which time, a capillary blood sample was taken from the finger and plasma lactate concentration
171 analyzed. The test was terminated when participants blood plasma lactate reached a value $\geq 4 \text{ mmol}\cdot\text{L}^{-1}$. Expired gas
172 samples were collected using the Douglas bag technique (Cranlea & Co. Bourneville, Birmingham, UK) in the last
173 minute of each exercise stage.

174 **Maximal Rate of Oxygen Uptake**

175 Maximal oxygen uptake ($\dot{V}O_{2\text{max}}$) was calculated following an incremental exercise test. The test began at 50 W for 4
176 minutes, and subsequent work rate increased by 30 W every minute until volitional exhaustion. The participants were
177 asked to maintain a pedalling cadence between 70 and 90 $\text{rev}\cdot\text{min}^{-1}$. A visual display in front of the participants was
178 used to maintain this cadence. Expired gas samples were collected using the Douglas bag technique and separate gas
179 samples were collected for a minimum of 3-minutes of before participants reached volitional exhaustion. The last
180 collection bag was only analyzed when collection time and expired volume was greater than 30 sec and 65 L,
181 respectively. Expired and inspired fractions of oxygen and carbon dioxide were determined with a gas analyzer
182 (Series 1400, Servomex, Crowborough, UK), calibrated using known gases (Linde Gas UK Ltd., West Bromwich,
183 UK), and expired volumes measured using a dry gas meter (Harvard Apparatus Ltd., Edenbridge, UK). A finger
184 prick capillary blood sample was taken four minutes after the end of the test and analysed for plasma lactate
185 concentration. All participants attained at least two of the following $\dot{V}O_{2\text{max}}$ criteria; 1) plateau in $\dot{V}O_2$ of $< 2.1 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$
186 between the last two gas collections, 2) blood plasma lactate $> 8 \text{ mmol}\cdot\text{L}^{-1}$, 3) respiratory exchange ratio \geq
187 1.15 (Howley and Bassett 1995).

188 **Submaximal Cycling Intensities**

189 The power to oxygen uptake (as a percentage of $\dot{V}O_{2\text{max}}$) relationship during the intermittent incremental exercise,
190 performed during visit 1, was used to establish power at 45, 55 and 65% of participants $\dot{V}O_{2\text{max}}$. Participants cycled at
191 each intensity for 10 minutes with a finger prick blood plasma sample measured 5 minutes into each stage (i.e. at 5,
192 15, 25 minutes of the protocol) with duplicate measurements averaged to provide blood plasma lactate and glucose.
193 Two, one-minute gas sample were collected between minutes 7-9 of each stage, and analyzed. Data collection of one
194 subject was stopped due to technical problems with the SRM ergometer during this part of the session.

195 Rates of whole-body carbohydrate and fat oxidation (i.e. CHox and FATox, respectively) were calculated based on
196 the following equations by Jeukendrup and Wallis (2005) for low (45% $\dot{V}O_{2max}$) and moderate intensity exercise (55
197 and 65% $\dot{V}O_{2max}$) with the assumption that protein oxidation during exercise was negligible:

$$\text{Fat Oxidation} = 1.695 * \dot{V}O_2 - 1.701 * \dot{V}CO_2$$

198 Low intensity (45% $\dot{V}O_{2max}$), Carbohydrate oxidation = $4.344 * \dot{V}CO_2 - 3.061 * \dot{V}O_2$

199 Moderate intensity (55 and 65% $\dot{V}O_{2max}$), Carbohydrate oxidation = $4.210 * \dot{V}CO_2 - 2.962 * \dot{V}O_2$

200 **16.1 km Cycling Time-Trial**

201 Participants completed 16.1 km time-trials on the SRM ergometer. As per manufactures instructions, the large
202 flywheel was attached to the ergometer to simulate kinetic energy as would be experienced during road cycling.
203 Participants could freely choose the cycling gear and cadence. The software program recorded power output, pedal
204 cadence, time and distance. Water was provided *ad libitum*. Participants received no temporal, verbal or
205 physiological feedback during the time-trial and were only aware of the distance they had covered. In order not to
206 interfere with the performance-based setting, no expired gas samples or blood samples were taken during the time-
207 trial. Immediately following the time-trial, participants rested passively and a blood sample for plasma lactate was
208 taken, with subsequent samples then taken every minute for the first 5 minutes, and then taken every 5 minutes for a
209 total of 15 minutes. Samples were analysed in duplicate and averaged.

210 **Statistical Analysis**

211 All statistical analyses were completed using SPSS 20.0 (SPSS, Chicago, IL). Data normality assumptions were
212 assessed using Kolmogorov-Smirnov test. Paired samples t-tests used were to compare physiological responses and
213 48 hours dietary intake between the supplement and placebo conditions. *A priori* power analysis showed a sample
214 size of 14 would allow detection of a 2-3% difference in 16.1 km time-trial performance with a high statistical power
215 ($1 - \beta = 0.80$; $0.05 = \alpha$ level). To determine the time-trial effect size, Cohen's *d* and subsequent power were
216 calculated (Cohen 1988). Differences between plasma lactate following the time-trial were analysed using a
217 condition (control vs. NZBC) by time-point (0, 1, 2, 3, 4, 5, 10, 15, and 20 min post time-trial) repeated measures
218 analysis of variance (ANOVA) with post-hoc t-tests. Mauchley's Test of Sphericity was conducted to test for
219 homogeneity of data and where violations were present Greenhouse-Geiser adjustments were made. All data are
220 reported as mean \pm SD and significance was set at alpha level of $P \leq 0.05$.

221

222 **RESULTS**223 **Blood Pressure, Lactate and Glucose in Rest**

224 Resting systolic blood pressure (NZBC: 124 ± 7 , PL: 123 ± 6 mmHg, $P = 0.556$), diastolic blood pressure (NZBC:
 225 79 ± 5 , PL = 78 ± 5 mmHg, $P = 0.190$), blood plasma lactate (NZBC: 1.15 ± 0.25 , PL: 1.02 ± 0.24 mmol·L⁻¹, $P =$
 226 0.23) and glucose (NZBC: 4.57 ± 0.45 , PL: 4.52 ± 0.44 mmol·L⁻¹, $P = 0.77$) were not different between conditions
 227 after 7-days of supplementation.

228

229 **Steady State Exercise, Energy Expenditure and Substrate Oxidation**

230 Across the three intensities, there were no differences between treatments in $\dot{V}O_2$, $\dot{V}CO_2$, heart rate, cycling
 231 economy, absolute power, blood plasma lactate, blood glucose or energy expenditure indicating that the participants
 232 experienced similar relative exercise intensities and physiological responses between treatments (Table 3). However,
 233 there were trends with NZBC for whole-body FATox rates to be 15 and 13% higher at 45% ($P = 0.077$) and 55%
 234 $\dot{V}O_{2max}$ ($P = 0.102$), but these were not matched by a significantly lower CHox rate ($P > 0.05$). At 65% $\dot{V}O_{2max}$,
 235 FATox was 27% higher following NZBC supplementation ($P = 0.044$), in line with a strong trend for lower CHox (P
 236 $= 0.06$). Correspondingly, the RER had a trend to be lower at 45% $\dot{V}O_{2max}$ ($P = 0.066$) and 55% $\dot{V}O_{2max}$ ($P = 0.120$).
 237 At 65% $\dot{V}O_{2max}$ RER was lower ($P = 0.043$) (Table 3).

238

239 **16.1 km Cycling Time-Trial Performance and Lactate Responses**

240 NZBC reduced 16.1 km completion time (NZ: 1678 ± 108 , PL: 1722 ± 131 sec, $P = 0.027$), with a group mean
 241 reduction of $2.4 \pm 3.7\%$ (range -2.7% - 8.7%) and 11 participants showing a decrease (Fig. 1). This was coupled with a
 242 trend for higher power across the time-trial (NZBC: 259 ± 29 , PL: 250 ± 33 W, $P = 0.155$) with no difference in
 243 heart rate (NZBC: 157 ± 14 , PL: 153 ± 15 beats·min⁻¹, $P = 0.247$) or cadence (NZBC: 92 ± 8 , PL: 93 ± 8 rev·min⁻¹, P
 244 $= 0.847$) between conditions. Post hoc effect size calculations indicate a 0.7 (medium-large) effect magnitude, with
 245 the achieved statistical power for the time-trial at 0.80. Absolute lactate values following the time-trial (Fig. 2)
 246 showed significant time ($F_{(1,13)} = 108.815$, $P < 0.001$) and condition effects ($F_{(1,13)} = 7.637$, $P = 0.016$) with between
 247 condition effects equating to 15% ($P = 0.003$), 10% ($P = 0.032$), 12% ($P = 0.004$), 11% ($P = 0.025$) and 15% ($P =$
 248 0.048) at 0, 2, 3, 4 and 15 minutes post time-trial, respectively, although there was no interaction effect ($F_{(1,13)} =$
 249 2.447 , $P = 0.191$).

250

251 **DISCUSSION**

252 This is the first study to observe that 7 days capsule intake of NZBC extract by trained endurance athletes enhanced
253 time-trial cycling performance by 2.4%. Intake of NZBC extract also increased whole-body FATox by 27% at
254 moderate intensity exercise ($\sim 65\% \dot{V}O_{2\max}$), which was coupled with a strong trend for lower whole-body CHOx ($P =$
255 0.06). A strong trend for higher whole-body FATox was also observed at low intensity exercise ($\sim 45\% \dot{V}O_{2\max}$, $P =$
256 0.077).

257

258 **Effects of NZBC extract on cycling time-trial performance**

259 Paton and Hopkins (2006) proposed that the “smallest worthwhile change” for road time-trial cyclists is around
260 0.6%. Our finding of a 2.4% increase in time-trial performance is considerably greater than this value and
261 comparable to other studies using supplements high in polyphenols, such as the 2.7% improvement in 16.1 km time-
262 trial following acute (~ 2.5 hours before time-trial) beetroot intake in male cyclists with similar $\dot{V}O_{2\max}$ values
263 (Lansley et al. 2011) and the 3.1% improvement in a 30 km time-trial with quercetin in elite cyclists (MacRae and
264 Mefferd 2006). Our finding of a 2.4% increase in time-trial performance represents a significant practical advantage
265 to athletes undertaking endurance exercise training because the performance increase occurred without alteration of
266 training or diet before the time-trial **and likely results from the trend for a higher power output across the time-trial**
267 **($P = 0.15$)**. In addition, all participants conformed to dietary restrictions and between experimental visits; there was
268 no difference in postprandial status as confirmed with resting glucose samples. The magnitude of the practical effect
269 of NZBC supplementation on 16.1 km performance can also be represented by using effect size statistic (Cohen
270 1988) and the calculated effect size for the present study of 0.7 indicates a moderate-large effect of NZBC extract
271 upon cycling time-trial performance. Participants did not report any change in frequency or type of their cycling
272 participation and reported to be participating in cycling exercise 8-10 hours a week during the 7-day supplementation
273 periods. In addition to using a randomised design, it is therefore unlikely the improvement in performance is
274 attributable to a chronic training effect of undertaking 7-days supplementation and exercise and therefore represents
275 a performance improvement achievable from a short duration (i.e. 7-days) intake. **However, with absence of markers**
276 **of phytochemical status in this study, that may be associated with the performance effect, we do not know whether a**
277 **shorter intake of NZBC results in similar performance improvements.**

278 A mechanism by which blackcurrant supplementation improves performance may involve improved endothelial
279 function. Anthocyanin-induced endothelium-dependant vasorelaxation of rat thoracic aorta is mediated by increased
280 production of endothelial-derived vasodilation factor nitric oxide (Nakamura et al. 2002). Delphinidin, a non-
281 glycoside anthocyanin, can also relax blood vessels by increasing nitric oxide through increased Ca^{2+} concentrations
282 in endothelial cells (Martin et al. 2002). Production of peroxynitrate from nitric oxide has also been shown to be
283 inhibited by polyphenols (Nagi et al. 2002). Blackcurrant containing a large amount of delphinidin and other
284 anthocyanins, therefore has the potential to increase peripheral blood flow by the combined action of increased nitric
285 oxide by endothelial cells and a reduced breakdown by nitric oxide free radicals. Indeed, an increase in peripheral
286 blood flow in typing work, a physical activity performed at a relatively very low intensity, following blackcurrant
287 intake has been reported (Matsumoto et al. 2005). Given the importance of nitric oxide in control of skeletal muscle
288 blood flow (Boushel et al. 2002) and potentially on skeletal muscle contractile efficiency (Bailey et al. 2010), it is
289 possible that such responses confer the performance benefits observed in the present study. **To elucidate such**
290 **mechanisms, future studies should examine the availability of nitric oxide and blood flow measures following NZBC**
291 **intake before, during and following exercise.**

292 **Following the time-trial, the significant affect for lactate across the 20-minute recovery period following NZBC may**
293 **represent alterations in production or removal of lactate through blood flow or changes in membrane lactate transport**
294 **mechanisms. However, in future studies on the effect of NZBC, measures of blood flow and lactate kinetics should**
295 **be examined during and following exhaustive exercise when lactate levels are typically elevated.**

296

297 **Effects of NZBC on substrate oxidation**

298 As far as we know, this is the first study to observe an improved FATox during moderate intensity cycling following
299 NZBC extract intake and is in contrast to previous work supplementing with quercetin (MacRae and Mefferd 2006).

300 In that study, no change in substrate oxidation was observed during a 30 km time-trial (MacRae and Mefferd 2006),

301 however, it needs to be acknowledged that no **substrate oxidation measures** were **obtained** during the time-trial in the

302 present study. Our increased fat oxidation at 65% $\dot{V}O_{2\max}$ from 0.37 ± 0.15 in the placebo condition to 0.44 ± 0.12

303 $\text{g}\cdot\text{min}^{-1}$ in the NZBC condition is similar in absolute values (i.e. $\text{g}\cdot\text{min}^{-1}$) and also magnitude of change to the FATox

304 rates observed during moderate intensity cycling following green-tea extract (Venables et al. 2008). An exact

305 comparison of studies with different polyphenols requires caution though as the possible variation in bioavailability

306 and subsequent interactions with the concomitant intake of other nutrients may affect observation (for a review see
307 Myburgh 2014). **In the present study, the observed alterations in substrate utilisation occurred following a**
308 **standardised absolute carbohydrate intake 2 hours before the event, with no alterations in circulating glucose or**
309 **hypoglycaemia (i.e. glucose < 3 mmol·L⁻¹) present during the exercise (Table 3).**

310 It is thought that lipolysis is not likely to limit whole-body FATox at the intensities used in the present study
311 (Horowitz et al. 1997) and it could be that blackcurrant has additional effects on lipid metabolism. **For example,**
312 chronic blackcurrant extract intake in C57BL/6J mice has been shown to elevate mRNA of genes involved with
313 energy expenditure including peroxisome proliferator-activated receptor alpha (Benn et al. 2014) and similarly,
314 Tsuda et al. (2005) observed that a total of 633 genes were up-regulated through treatment of rat adipocytes with
315 cyanidin-3-glycoside, which included genes involved in lipid metabolism and signal transduction-related genes.
316 **Therefore,** the increased whole-body FATox may result from a combination of many pathways acting synergistically
317 including up regulation of genes for proteins involved in FATox, transport of fatty acids into mitochondria, improved
318 nitric oxide availability and increased peripheral blood flow.

319 **Limitations**

320 Participants were allowed to consume their normal diet 46 hours before the testing sessions (except the dietary
321 restrictions such as caffeine on the day, alcohol the day before and the standard breakfast 2 hours before the session)
322 and participants were instructed to use a recorded food diary from the third visit (i.e. 1st condition visit) and replicate
323 this for the cross-over condition visit. Due to the wide availability of polyphenols within normal dietary intake,
324 participants were not restricted in their choice of foods, therefore it cannot be ruled out that some participants may
325 have consumed more polyphenols in the 48-hour period. We also did not measure the antioxidant status and were not
326 able to quantify polyphenol or anthocyanin intake of participants. This therefore will not highlight if there were any
327 intra and inter differences in phytochemical status of participants and account that activity of anthocyanins can be
328 synergistically or antagonistically altered by other phytochemicals and vitamins found in fruits (Niki et al. 1998). In
329 addition, it should also be recognised that a food diary collected from the first experimental condition and replicated
330 in the second experimental condition has disadvantages such as a large variability in food intake between participants
331 and the intake recorded the first time and then replicated may not represent an appropriate or optimal intake for that
332 participant (Jeacocke and Burke 2010). **It is also accepted that the use of a standardised breakfast of one slice of toast**
333 **or bread 2 hours before the start of testing does not represent a typical pre-race condition (i.e. < 1g·kg body mass⁻¹).**

334 However, the intake of carbohydrate before measurement of substrate utilisation required standardisation due to the
335 affect intake of carbohydrate before exercise can have on substrate utilisation (Achten and Jeukendrup 2003).

336 With a 7-day NZBC supplementation representing a nutritional ergogenic aid (as in the present study), we
337 do not know the time - and dose-dependent metabolic, physiological and performance effects of NZBC extract
338 intake. Our daily dose of 105 mg·day⁻¹ was according to manufacturers guidelines and the supplementation period in
339 line with previous studies using berry juices also applying multiple days of intake before exercise test (e.g. Connolly
340 et al. 2006; Howatson et al 2010; Bowtell et al. 2011). Wu et al. (2004) estimated that the average anthocyanin intake
341 in U.S. adults as 12.5 mg·day⁻¹. Our daily dose of anthocyanin from NZBC extract capsules was approximately 8
342 times higher than this, but is considerably lower than other studies using polyphenol supplements such as 1000
343 mg·day⁻¹ of quercetin (Cureton et al. 2009). In that study, the participants did not report any side-effects; however,
344 the minimum dose and duration of NZBC extract needed to elicit ergogenic effects are unknown. Future studies
345 should therefore examine dosing strategies of NZBC with emphasis on elucidating the optimal dose, frequency and
346 duration of intake.

347 **Conclusions**

348 **Short-term (7-days)** intake of NZBC extract capsules is associated with an **improved** 16.1km time-trial cycling
349 performance **obtained with higher plasma lactate values, and an increased** whole-body fat oxidation at moderate
350 intensity exercise (~65% $\dot{V}O_{2max}$). These findings may have implications for nutritional strategies used by endurance
351 athletes to enhance performance and alter substrate utilisation.

352

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356

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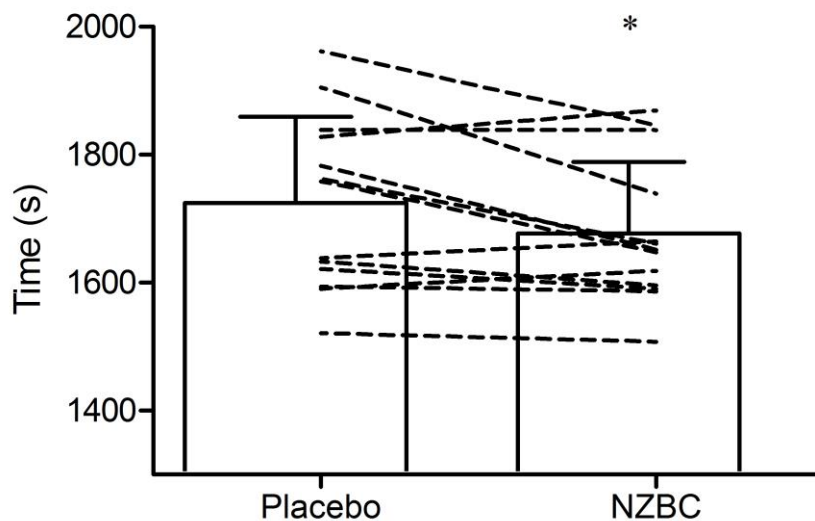
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448 FIGURE LEGENDS

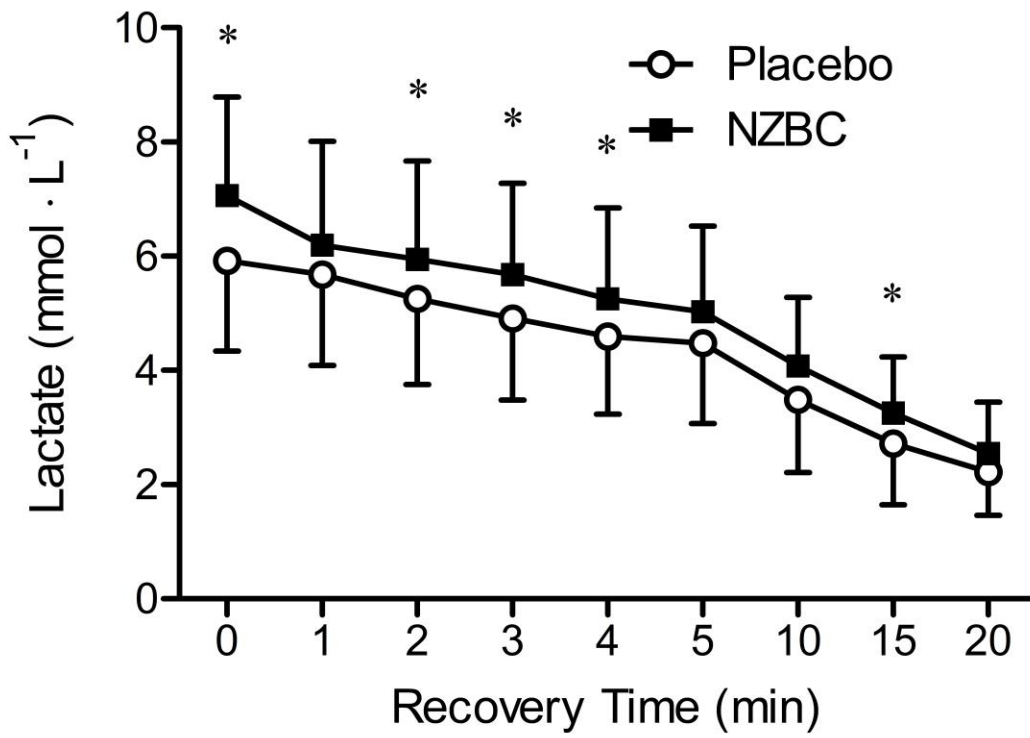
449 **Fig. 1** Exercise time of the 16.1 km time-trial. Columns show group mean \pm SD. Dashed lines show the individual
450 responses. *Completion time was reduced after NZBC extract ($P < 0.05$).

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Fig. 2 Blood plasma lactate across 20-minute passive recovery following the 16.1 km time-trial after NZBC (filled circles) and placebo (open circles). Data are mean \pm SD. * denotes significant difference between groups ($P < 0.05$).



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471 **Table 1. Participant characteristics**

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Age (years)	38±13
Height (cm)	178±4
Body Mass (kg)	77±9
$\dot{V}O_{2\max}$ (mL·kg ⁻¹ ·min ⁻¹)	53±6
$\dot{V}O_{2\max}$ (L·min ⁻¹)	4.1±0.5
RER _{max}	1.17±0.07
Power (Lactate 4 mmol·L⁻¹) (W)	290±26
Lactate _{max} (mmol·L ⁻¹)	7.51±0.81
Heart Rate _{max} (beats·min ⁻¹)	182±12
WR _{max} (W)	366±36
% Body Fat	13.7±2.6

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474 Maximum values were collected during the incremental maximal cycling test to volitional
 475 exhaustion. $\dot{V}O_{2\max}$, maximum rate of oxygen uptake; RER_{max}, maximum respiratory exchange ratio;
 476 **Power (Lactate 4 mmol·L⁻¹)**, power that elicits a plasma lactate of 4 mmol·L⁻¹ measured during an
 477 **intermittent incremental cycling test**; Lactate_{max}, maximum lactate value achieved four minutes after the
 478 end of the test; Heart Rate_{max}, maximum heart rate; WR_{max}, maximum work rate. Data reported as mean ±
 479 SD from 14 participants.

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482 **Table 2. Absolute and relative to body mass dietary intake 48 hours before experimental**483 **visits.**

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	Placebo	NZBC
Carbohydrate (g)	474±117	460±150
(g·kg body mass ⁻¹)	6.3±2.4	6.3±2.9
Fats (g)	150±60	159±53
(g·kg body mass ⁻¹)	2.0±1.0	2.0±0.9
Protein (g)	179±48	180±42
(g·kg body mass ⁻¹)	2.2±0.8	2.2±0.8
Total Energy Intake (kJ)	16544±3390	16590±3818
(kJ·body mass ⁻¹)	204.9±77.9	206.8±86.9

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486 Data reported as mean ± SD from 14 participants.

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491 **Table 3. Data during submaximal cycling at low (45 & 55% $\dot{V}O_{2max}$) and moderate intensities (65%**
 492 **$\dot{V}O_{2max}$).**

Variable	45% $\dot{V}O_{2max}$		55% $\dot{V}O_{2max}$		65% $\dot{V}O_{2max}$	
	Placebo	NZBC	Placebo	NZBC	Placebo	NZBC
Power (W)	121±16	122±16	160±18	159±17	198±21	199±20
$\dot{V}O_2$ (L·min ⁻¹)	1.80±0.19	1.79±0.21	2.17±0.22	2.21±0.25	2.68±0.22	2.70±0.23
$\dot{V}CO_2$ (L·min ⁻¹)	1.62±0.21	1.60±0.22	1.97±0.23	1.99±0.26	2.43±0.28	2.42±0.26
Relative Intensity (% $\dot{V}O_{2max}$)	44±2	44±4	54±4	55±5	66±4	67±4
Cycling Economy (mL·kg ⁻¹ ·W ⁻¹)	11.5±1.4	11.5±1.4	10.7±1.2	11.0±1.2	10.6±1.3	10.7±1.2
Heart rate (beats·min ⁻¹)	105±11	106±11	117±12	118±13	132±14	132±15
Lactate (mmol·L ⁻¹)	1.05±0.29	1.01±0.26	0.92±0.29	0.88±0.19	1.19±0.49	1.09±0.29
Glucose (mmol·L⁻¹)	4.25±0.43	4.27±0.67	4.01±0.58	4.08±0.56	4.14±0.67	4.05±0.60
Energy Expenditure (kJ·min ⁻¹)	36±7	35±8	43±9	44±10	53±11	54±11
CH _{ox} (g·min ⁻¹)	1.6±0.39	1.52±0.40	1.85±0.43	1.80±0.43	2.36±0.54	2.23±0.48
FAT _{ox} (g·min ⁻¹)	0.26±0.1	0.29±0.09	0.33±0.14	0.38±0.09	0.37±0.15	0.44±0.12*
RER	0.91±0.04	0.90±0.04	0.91±0.04	0.89±0.03	0.91±0.04	0.90±0.03*

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 495 All measures were collected following 7 days supplementation with NZBC extract during steady state
 496 cycling, and 2 hours post-prandial of a standard low calorie carbohydrate breakfast (1 slice of bread and
 497 the last capsule). CH_{ox}, carbohydrate oxidation; FAT_{ox}, fat oxidation; **RER**, respiratory exchange ratio.
 498 Data reported as mean ± SD from 13 participants. * denotes $P < 0.05$ vs. placebo.
 499