

As accepted for publication, <http://journals.humankinetics.com/ijnsnem>

1 Beneficial physiological effects with blackcurrant intake in endurance athletes

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12 Running head: Blackcurrant and exercise-mediated effects

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## 19 **ABSTRACT**

20 Blackcurrant contains anthocyanins, known to influence vasorelaxation and peripheral blood

21 flow. We examined the effects of 7 days intake of Sujon New Zealand blackcurrant powder

22 (6g/day) on the lactate curve, maximum oxygen uptake, and cardiovascular responses at rest and

23 during cycling. Thirteen trained triathletes with >3 yrs experience (8 men, age: 38±8 yrs, body

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24 mass:  $71 \pm 9$  kg, BF%:  $19 \pm 5\%$ , mean  $\pm$  SD) performed two incremental cycling protocols with  
25 recording of physiological and cardiovascular responses (Portapres® Model 2). Cardiovascular  
26 function was also measured in rest. Experimental design was double-blind, placebo-controlled,  
27 randomized and cross-over (wash-out 4 wks). Data was analysed with two-tailed t-tests and 2-  
28 way ANOVA and significance accepted at  $p < 0.05$ . Plasma lactate was lower at 40%, 50%, 60%  
29 and 70% of maximum power by 27%, 22%, 17% and 13%. Intensity at  $4 \text{ mmol} \cdot \text{L}^{-1}$  OBLA was  
30 6% higher with blackcurrant without effect on heart rate and oxygen uptake. Maximum values of  
31 oxygen uptake, heart rate and power were not affected by blackcurrant, but obtained with 14%  
32 lower lactate. In rest, blackcurrant increased stroke volume and cardiac output by 25% and 26%,  
33 and decreased total peripheral resistance by 16%, with no changes in blood pressure and heart  
34 rate. Cardiovascular responses during exercise at 40%, 50%, 60%, 70% and 80% intensity were  
35 not affected. Sujon New Zealand blackcurrant powder affects lactate production and/or clearance  
36 during exercise. Sujon New Zealand blackcurrant powder affects physiological and  
37 cardiovascular responses in rest and during exercise that may have implications for exercise  
38 performance.

39  
40 Key words: Plasma lactate; cardiovascular function, New Zealand blackcurrant

## 41 42 **INTRODUCTION**

43  
44 Blackcurrant is a rich source for anthocyanin, a flavonoid that has attracted attention  
45 recently for human health benefits such as reduced risks for myocardial infarction (Cassidy et al.,  
46 2013) and type 2 diabetes (Wedick et al., 2012), inhibition of the proliferation of cancer cells

47 (Bishayee et al., 2010), anti-inflammatory effects (Zhu et al., 2013), and anti-oxidant activity (De  
48 la Cruz et al., 2013). Ergogenic aids with anti-inflammatory and anti-oxidant activity counteract  
49 potentially negative responses to exercise. For example, catechins, shiitake, resveratrol,  
50 quercetin, montmorency cherries, that are known ergogenic aids with anti-inflammatory and  
51 anti-oxidant properties, have been examined primarily for their effectiveness on exercise  
52 recovery (Haramizu et al., 2011; McAnulty et al., 2013; Zembron-Lacny et al., 2013). In  
53 addition, anthocyanin-containing fruits and berries, including blackcurrant, were examined for  
54 post-exercise effects on immune and functional responses (montmorency cherry juice: Bowtell et  
55 al., 2011; tart cherry juice: Connolly et al., 2006; blackcurrant extract: Lyall et al., 2009;  
56 blueberry: McLeay et al., 2012). It is not known whether the effects are caused by intake of a  
57 total amount of anthocyanins or amount of specific anthocyanins. However, the effectiveness of  
58 such ergogenic aids on the physiological responses during exercise has not been addressed.

59 Blackcurrant has an effect on human cardiovascular responses during activity. Peripheral  
60 blood flow was increased by 22% during typing work in humans (Matsumoto et al., 2005),  
61 potentially by anthocyanin-induced vasorelaxation and vasodilation as shown in thoracic aortic  
62 rings in male Wistar rats (Zibera et al., 2013). In intact rat aortic rings (i.e. with functional  
63 endothelium), anthocyanins induced relaxation by 37%, possibly by involvement of the  
64 purinergic pathway producing nitric oxide (Mendes et al., 2003). Edirisinghe et al (2011)  
65 provided evidence using human umbilical vein endothelial cells that blackcurrant concentrate  
66 activated eNOS via the Akt/PI3 kinase pathway. In addition, Nakamura et al (2002) observed  
67 that blackcurrant concentrate enhanced the synthesis of nitric oxide and induced dose-dependent  
68 relaxation up to 80%. In this study, removal of the endothelium blunted the effect of the  
69 anthocyanins, supporting endothelial function to be affected. Caution is required, however, to

70 generalize from in vitro exposure of arteries with blackcurrant to an in-vivo condition in humans.  
71 However, dietary intake of anthocyanin intake was associated with lower arterial stiffness and  
72 central blood pressure in women (Jennings et al., 2012). All together, these studies provide the  
73 evidence that may support an effect of anthocyanin on cardiovascular responses in rest and  
74 during exercise.

75 An anthocyanin effect on cardiovascular responses during exercise may influence the  
76 delivery of substrates and removal of metabolic products of skeletal muscles. It is a classic  
77 observation, for example, that during exercise of incremental intensity, blood lactate accumulates  
78 in an exponential fashion as a consequence of production and removal mechanisms. An increase  
79 in peripheral blood flow by anthocyanin may benefit the removal mechanisms of lactate as  
80 uptake may be enhanced by liver, heart, kidney and skeletal muscles. As such, intracellular  
81 lactate oxidation (Gladden, 2008) and lactate conversion into glucose may be enhanced and  
82 could potentially lead to glycogen sparing (Emhoff et al., 2013). Increases in peripheral blood  
83 flow may also affect the oxygen consumed during exercise and potentially reduce effects of  
84 peripheral fatigue mechanisms and beneficial for exercise performance. The effects of  
85 blackcurrant on the lactate responses during incremental exercise and maximum oxygen uptake  
86 have not been examined.

87 Lactate threshold or absolute lactate values during incremental exercise and maximum  
88 oxygen uptake are recognized indicators of the ability for endurance performance [for a review  
89 see (Bentley et al., 2007)]. An increased peripheral blood flow may affect lactate clearance and  
90 oxygen delivery influencing the exercise intensity at lactate indicators (e.g. onset of blood lactate  
91 accumulation (OBLA) at  $4 \text{ mmol}\cdot\text{L}^{-1}$ ), maximum oxygen uptake and the cardiovascular  
92 responses in rest and during exercise. Therefore, the primary objective was to examine the effect

93 of 7 days supplementation with Sujon New Zealand blackcurrant powder on the blood plasma  
94 lactate curve and aerobic capacity of trained triathletes. Second objective was to examine  
95 whether such supplementation would affect the cardiovascular responses at rest and exercise.

96

## 97 **METHODS**

98

99 Healthy male (n=8) and female (n=5) triathletes with >3 yrs triathlon experience (age: 38±8 yrs,  
100 height: 174±5 cm, body mass: 71±9 kg, BMI: 23±2, BF%: 19±5%, mean±SD) were recruited  
101 from local triathlon clubs and volunteered without payment. Participants provided written  
102 informed consent after explanation of the experimental procedures, potential risks and benefits.  
103 Ethical approval was obtained from the University of Chichester Ethics Research Committee. In  
104 brief, participants visited for one familiarization and two experimental testing sessions.  
105 Familiarization consisted of practicing all experimental procedures and recording of baseline  
106 subject characteristics [i.e. age, height, body mass, BMI, body fat (%) (Tanita BC418 segmental  
107 body composition analyser)]. Participants attended for two experimental visits to perform cycling  
108 protocols to examine the effectiveness of 7 days of intake of Sujon New Zealand blackcurrant  
109 powder [NZBC, 6g/day (138.6 mg anthocyanins) or placebo (PBO)]. Placebo was a  
110 commercially available blackcurrant juice with British blackcurrants containing likely about 3-4  
111 mg anthocyanins per dose (Mattila et al., 2011). We were not able to quantify the bioavailability  
112 of anthocyanins in the blood. Optimal dosing strategy of New Zealand blackcurrant powder is  
113 not known and the dose was according manufacturers guidelines. Studies on the effectiveness of  
114 berry juices applied also multiple days of intake before exercise testing (e.g. 8 days: Bowtell et  
115 al., 2011; 4 days: Connolly et al., 2006; 6 days: Howatson et al., 2010). The experimental design

116 was double blind, randomized, placebo-controlled, and cross-over with drinks provided in  
117 unlabelled bottles. Participants recorded their dietary intake for 48 hr before attending the first  
118 experimental visit and followed the identical dietary pattern before the second visit. Participants  
119 were instructed not to exercise and consume alcohol 24 hr before each visit, be well-rested and  
120 hydrated on arrival, and not take other supplements that add further nutritional value to the  
121 normal diet. For the experimental visits, participants visited 2-3 hr postprandial, after a light  
122 breakfast of toast and water and intake of the final supplement. All testing occurred in a  
123 temperature controlled ( $\sim 18^{\circ}\text{C}$ ) exercise physiology laboratory. The sequence of testing during  
124 an experimental visit comprised of 20 min recording of resting cardiovascular function followed  
125 by the cycling protocol for lactate responses with recording of physiological and cardiovascular  
126 responses. Then, following a 30 min rest, the cycling protocol for maximum oxygen uptake was  
127 performed.

128

129 Experimental procedures

130 *Incremental cycling protocols*

131 The incremental cycling protocol for lactate responses consisted of 4 min stages with 2  
132 min recovery, start power 50 W with 30 W increments [adapted from (González-Haro et al.,  
133 2007)], with termination by obtaining a plasma lactate response close to or over  $4.0 \text{ mmol}\cdot\text{L}^{-1}$ .

134 The maximum oxygen uptake protocol had a start power 50 W for 4 min with  $30 \text{ W}\cdot\text{min}^{-1}$   
135 increments [adapted from (Bailey et al., 2009)] with termination by voluntary exhaustion.

136 Cycling protocols were performed at self-selected pedal cadence (70-90 rpm) on an ergometer  
137 (SRM ergometer, SRM International, Germany). In the last minute of each stage during the  
138 cycling protocol for lactate, expired air was collected using the Douglas bag technique and heart

139 rate (Polar Vantage NV, Polar Electro Oy, Kempele, Finland) recorded. Expired air was analysed  
140 with three-point calibrated Servomex gas analysers (Servoflex MiniMP, 5200 Multipurpose) and  
141 volume measured (Harvard dry gas meter). Gas volumes were calculated using Haldane  
142 transformation and standardisation to STPD conditions with consideration of fractions of oxygen  
143 and carbon dioxide of inspired air. Blood samples for analysis of plasma lactate were taken using  
144 finger-prick method immediately after each stage and 3 min after voluntary exhaustion during  
145 the maximum oxygen uptake test (2300 STAT Plus™ analyser, YSI Life Sciences, Yellow  
146 Springs, USA). In at least the last 4 minutes of the maximum oxygen uptake protocol, expired  
147 gases were collected in 45 seconds samples with 30 seconds samples in the last minute using  
148 Douglas bags (Plysu Protection Systems Limited, Milton Keynes, UK). A blood sample was  
149 taken 3 minutes after the end of the test.

150

#### 151 *Cardiovascular measurements*

152 Cardiovascular responses were recorded using a beat-to-beat blood pressure monitoring  
153 system (Portapres® Model 2, Finapres Medical Systems BV, Amsterdam, The Netherlands) in  
154 rest and during the incremental cycling protocol for lactate responses. The Portapres® Model 2 is  
155 a beat-to-beat finger pressure analyser that allows the non-invasive continuous measurement of  
156 hemodynamic parameters. The Portapres has shown reliability for the relative changes in cardiac  
157 output (Sugawara et al., 2003). Clear outliers in recorded data were removed. The finger cuff  
158 was positioned around the same finger of the hand of the left arm. For the resting condition,  
159 participants were in a supine position for 20 min. Cardiovascular measures in rest were averaged  
160 over 10 consecutive beats, with the lowest systolic blood pressure (BP) and associated measures  
161 analysed. For the exercise condition, cardiovascular measures were averaged for the last minute

162 of each stage in which participants held the left arm in front of their chest to enable recording of  
163 the signal with less noise. The following parameters were derived: stroke volume, cardiac output,  
164 systolic blood pressure, diastolic blood pressure, mean arterial pressure, and total peripheral  
165 resistance (Beatscope 1.1a., Finapres Medical Systems BV, Amsterdam, The Netherlands).

166

### 167 *Supplementation protocol*

168 Participants were provided with a 4 day supply of Sujon New Zealand blackcurrant powder (24  
169 gram in 571 mL of water) followed by 3 day supply (18 gram in 429 mL of water), dissolved in  
170 opaque bottles. Sujon New Zealand blackcurrant powder contains 138.6 mg anthocyanin, 49 mg  
171 Vit C and 5.2 gram of carbohydrates per 6 gram serving. Per serving, total phenolic content was  
172 271.6 mg. Placebo was a commercially available low-calorie blackcurrant juice drink (Ribena  
173 Blackcurrant), containing 32 mg Vit C and ~1.6 gram of carbohydrates per 250 ml serving.  
174 Participants were instructed to take the supplement or placebo with breakfast with the last intake  
175 about 2 hrs before an experimental visit. Participants were provided with a marked plastic cup to  
176 ensure equal daily intake over a 7-day period. Wash-out period was 4 weeks (Jin et al., 2011).

177

### 178 *Statistical analysis.*

179 The mathematical relationship between cycling power and lactate for each individual was  
180 determined with a third degree polynomial using lactate analysis software (Newell et al., 2007)  
181 [ $R^2$  values of  $0.9868 \pm 0.0174$  (placebo) and  $0.9871 \pm 0.0198$  (Sujon New Zealand blackcurrant  
182 powder)] and calculated at 30%, 40%, 50%, 60%, 70% and 80% of maximal cycling power  
183 obtained with the maximum oxygen uptake cycling test. Intensity, oxygen uptake and heart rate  
184 were calculated at a lactate rise of  $1 \text{ mmol} \cdot \text{L}^{-1}$  and a lactate value of  $4 \text{ mmol} \cdot \text{L}^{-1}$  (OBLA, onset of



185 blood plasma lactate accumulation) using the lactate analysis software. Cardiovascular responses  
186 during exercise were taken for the stage closest to 40%, 50%, 60%, 70% and 80% of maximum  
187 oxygen uptake resulting in intensity for placebo of  $41\pm 5\%$ ,  $51\pm 4\%$ ,  $60\pm 6\%$ ,  $70\pm 3\%$ , and  $79\pm 4\%$   
188 and for the Sujon New Zealand blackcurrant powder condition  $41\pm 3\%$ ,  $48\pm 2\%$ ,  $57\pm 3\%$ ,  $67\pm 6\%$ ,  
189 and  $80\pm 4\%$ . Data for 3 participants for the cardiovascular responses during exercise was  
190 excluded due to the inability of recording of a clean signal. A sample size of 9 would allow  
191 detection of a 20% difference in cardiac output at rest (power: 0.80:  $\alpha$ -level: 0.05). Resting  
192 cardiovascular data passed normality check with D'Agostino and Pearson omnibus normality test  
193 (Prism v5.04, Graphpad Software Inc.) to allow paired t-tests. Lactate and cardiovascular  
194 responses during exercise were analysed with 2-way ANOVA and post hoc t-tests to examine  
195 condition effects at each intensity. Significance was accepted at  $p < 0.05$ .

196

## 197 **RESULTS**

### 198 *Lactate responses*

199 The intensity at  $1 \text{ mmol}\cdot\text{L}^{-1}$  lactate rise was 4% higher with Sujon New Zealand blackcurrant  
200 powder (PBO:  $184\pm 52$ , NZBC:  $192\pm 52$  W, range -2 to 14%, 8 participants showed an increase  
201 and 1 no change) ( $p=0.02$ ). In both conditions at a  $1 \text{ mmol}\cdot\text{L}^{-1}$  lactate rise, there were no  
202 differences in heart rate (PBO:  $141\pm 16$ , NZBC:  $141\pm 14 \text{ b}\cdot\text{min}^{-1}$ ,  $p=0.83$ ) or oxygen uptake  
203 (PBO:  $2.54\pm 0.66$ , NZBC:  $2.49\pm 0.63 \text{ L}\cdot\text{min}^{-1}$ ,  $p=0.41$ ). The intensity at  $4 \text{ mmol}\cdot\text{L}^{-1}$  OBLA was  
204 6% higher with Sujon New Zealand blackcurrant powder (PBO:  $223\pm 57$ , NZBC:  $236\pm 60$  W,  
205 range -5 to 22%, 11 participants showed an increase and 1 no change) ( $p=0.007$ ) (Figure 1). In  
206 both conditions at  $4 \text{ mmol}\cdot\text{L}^{-1}$  OBLA, there were no differences in heart rate (PBO:  $159\pm 7$ ,  
207 NZBC:  $164\pm 10 \text{ b}\cdot\text{min}^{-1}$ ,  $p=0.13$ ) or oxygen uptake (PBO:  $2.91\pm 0.73$ , NZBC:  $2.96\pm 0.71 \text{ L}\cdot\text{min}^{-1}$ ,

208  $p=0.31$ ). Plasma lactate was lower at 40% (PBO:  $1.24\pm 0.52$ , NZBC:  $0.91\pm 0.46$   $\text{mmol}\cdot\text{L}^{-1}$ ,  
209  $p=0.001$ ), 50% (PBO:  $1.58\pm 0.78$ , NZBC:  $1.23\pm 0.64$   $\text{mmol}\cdot\text{L}^{-1}$ ,  $p=0.002$ ), 60% (PBO:  $2.29\pm 0.96$ ,  
210 NZBC:  $1.91\pm 0.87$   $\text{mmol}\cdot\text{L}^{-1}$ ,  $p=0.001$ ) and 70% (PBO:  $3.52\pm 1.10$ , NZBC:  $3.08\pm 1.21$   $\text{mmol}\cdot\text{L}^{-1}$ ,  
211  $p=0.004$ ) of maximum power, decreases of 27%, 22%, 17% and 13%, respectively (Figure 2).

212

### 213 *Maximum oxygen uptake*

214 There was no effect on maximum values of oxygen uptake (PBO:  $49.1\pm 6.2$ , NZBC:  $49.7\pm 6.1$   
215  $\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ,  $p=0.16$ ), power (PBO:  $305\pm 68$ , NZBC:  $307\pm 62$  W,  $p=0.66$ ) or heart rate (PBO:  
216  $172\pm 10$ , NZBC:  $172\pm 11$   $\text{b}\cdot\text{min}^{-1}$ ,  $p=0.68$ ). However, maximum oxygen uptake with Sujon New  
217 Zealand blackcurrant powder was obtained with 14% lower lactate values (measured 3-min after  
218 exhaustion; PBO:  $7.85\pm 1.69$ , NZBC:  $6.79\pm 1.51$   $\text{mmol}\cdot\text{L}^{-1}$ , range -27 to 48%, 10 participants  
219 showed a decrease and 1 no change) ( $p=0.02$ ) (Figure 3).

220

### 221 *Cardiovascular function in rest*

222 There were no differences in systolic BP (PBO:  $121\pm 23$ , NZBC:  $120\pm 23$  mmHg,  $p=0.92$ ),  
223 diastolic BP (PBO:  $69\pm 16$ , NZBC:  $63\pm 14$  mmHg,  $p=0.12$ ), mean arterial BP (PBO:  $86\pm 18$ ,  
224 NZBC:  $82\pm 18$  mmHg,  $p=0.33$ ), and heart rate (PBO:  $58\pm 9$ , NZBC:  $59\pm 10$   $\text{beats}\cdot\text{min}^{-1}$ ,  $p=0.95$ ).  
225 Stroke volume (PBO:  $82\pm 23$ , NZBC:  $99\pm 25$  mL,  $p=0.006$ ) and cardiac output (PBO:  $4.8\pm 1.6$ ,  
226 NZBC:  $5.8\pm 1.7$   $\text{L}\cdot\text{min}^{-1}$ ,  $p=0.015$ , Figure 4A) were increased by 25% and 26%, respectively.  
227 There was a 16% lower total peripheral resistance (PBO:  $20.2\pm 8.9$ , NZBC:  $15.2\pm 5.3$   $\text{mmHg}\cdot\text{L}^{-1}$   
228  $\cdot\text{min}^{-1}$ ,  $p=0.05$ ) (Figure 4B). The changes in resting cardiovascular function were observed in 10  
229 participants.

230

231 *Cardiovascular responses during exercise*

232 At each intensity, there were no differences in diastolic BP ( $p=0.56$ ), systolic BP ( $p=0.76$ ), mean  
233 arterial blood pressure ( $p=0.54$ ), heart rate ( $p=0.78$ ), stroke volume ( $p=0.88$ ), cardiac output  
234 ( $p=0.97$ ) and total peripheral resistance ( $p=0.58$ ) between placebo and New Zealand blackcurrant  
235 at each intensity (Table 1).

236

237 **DISCUSSION**

238

239 The present study provides evidence for an effect on the physiological responses during  
240 exercise and resting cardiovascular function by short duration (1 week) intake of an anthocyanin-  
241 containing supplement. Short-term supplementation with Sujon New Zealand blackcurrant  
242 powder shifted the lactate curve during exercise, allowed maximum oxygen uptake with lower  
243 lactate, increased resting cardiac output and stroke volume and decreased resting total peripheral  
244 resistance. No effects were observed for cardiovascular responses during exercise. In addition,  
245 no effects were observed for functional capacity (i.e. power at maximum oxygen uptake), thus  
246 the practical implications of the supplement intervention are still unclear.

247 As far as we know, the substantial combined downward and rightward shift of the lactate  
248 curve in the present study with Sujon New Zealand blackcurrant powder has not been reported  
249 by other supplement intake. It is well known that the downward and rightward shift of the lactate  
250 curve results from physiological and metabolic adaptations by endurance training (Faude et al.,  
251 2009) and high-intensity training (Evertsen et al., 2001); in our study, the right-ward shift of the  
252 lactate curve resulted that OBLA was obtained at a 6% higher cycling intensity. A delay in  
253 OBLA may have beneficial implications for endurance athletes as it would allow prolonged high

254 intensity exercise during competition and endurance exercise training. In addition, a delay in  
255 OBLA could be predictive for an enhancement of cycling endurance performance. However,  
256 supplementation with Sujon New Zealand blackcurrant powder did not replicate other common  
257 endurance training adaptations in physiological responses because heart rate and oxygen uptake  
258 at OBLA, for example, were not affected. Other supplements have been reported to be able to  
259 shift the lactate curve right-ward. For example, recreationally active men delayed OBLA after 28  
260 days of beta-alanine supplementation but with increased absolute heart rate as a percentage of  
261 maximum values (Jordan et al., 2010). In endurance-trained cyclists, OBLA with beta-hydroxy  
262 betamethylbutarate and leucine was delayed by 9.1% and 2.1%, respectively (Vukovich &  
263 Dreifort, 2001). Neither of these studies reported a downward shift of the lactate curve.  
264 Therefore, the mechanisms causing delayed OBLA in previous studies by supplementation  
265 (Jordan et al., 2010) or training (Evertsen et al., 2001) are possibly explained by different  
266 physiological mechanisms that delayed the OBLA and blood lactate accumulation in the present  
267 study. Blood lactate accumulation results from an imbalance between lactate appearance and  
268 removal mechanisms (Brook, 1985). Our data provides evidence for the following acute  
269 physiological adaptations with short-term anthocyanin supplementation. First, short-term  
270 supplementation with anthocyanin seems to alter the balance of lactate appearance and removal  
271 mechanisms to blood lactate accumulation. Second, it seems that the alteration of the balance of  
272 factors contributing to blood lactate accumulation is/are intensity-dependent with exercise at  
273 relatively low intensity to be more affected. The lactate appearance may have been altered by an  
274 anthocyanin effect on substrate oxidation such that there was potentially an increased  
275 contribution of fat oxidation at relatively low intensities. Future work should address whether  
276 substrate oxidation is altered with blackcurrant supplementation. The removal mechanisms may

277 have been enhanced by an increase in peripheral blood flow. An increase in peripheral blood  
278 flow was reported by Matsumoto et al (2005), in typing work, a physical activity performed at  
279 relatively low intensity. We were not able to quantify the contribution of lactate appearance and  
280 removal mechanism towards reduced lactate at low and moderate exercise intensities. However,  
281 increased peripheral blood flow by anthocyanin vasodilating effects may have contributed to  
282 increased lactate clearance.

283         Maximum oxygen uptake was not affected by blackcurrant intake but obtained with  
284 lower lactate values. Although the protocols for obtaining the lactate curve and maximum  
285 oxygen uptake were different in stage duration, the lower lactate value may indicate reduced  
286 contribution of anaerobic glycolysis needed to reach exhaustion or increased clearance  
287 immediately following exercise and being at rest.

288         At rest (but without preceding exercise), total peripheral resistance was reduced with  
289 blackcurrant supplementation. As far as we know, there is no evidence that anthocyanins or their  
290 metabolites can reduce sympathetic drive, but if this had happened, resting heart rate would have  
291 been affected and that was not the case. In humans, it was observed that orally provided  
292 anthocyanins (320 mg containing 17 different anthocyanins from bilberry and blackcurrant)  
293 induced an increase in brachial flow-mediated dilation of about 10% 2hr after intake in  
294 hypercholesterolemic individuals (Zhu et al., 2011). The dose in our study was 138.6 mg of total  
295 anthocyanins in Sujon New Zealand blackcurrant powder. In addition, Zhu et al (2011) provided  
296 the anthocyanin in capsule form bypassing possible anthocyanin-dependent degradation in mouth  
297 saliva (Kamonpatana et al., 2012; Kamonpatana et al., 2014) by powder intake in the present  
298 study, although potential effects are small due to the time needed for degradation by saliva. It is  
299 possible that in our study, blackcurrant anthocyanins (or its metabolites) increased nitric oxide

300 production (Xu et al., 2004; Zibera et al., 2013) leading to a decrease in total peripheral  
301 resistance. However, the reciprocal changes of reduced total peripheral resistance and increased  
302 cardiac output (by increased stroke volume), but no changes in resting heart rate and blood  
303 pressure suggests probably more complex hemodynamic mechanisms. For example, increased  
304 resting cardiac output could normally result from an increase in resting heart rate but this was not  
305 the case.

306         There are some limitations of the present study. First, our understanding of the  
307 mechanisms of the effects by oral intake of Sujon New Zealand blackcurrant powder requires  
308 information on the bioavailability of anthocyanins and metabolites. In addition, work is required  
309 to examine the mechanisms of physiological effects by anthocyanins and metabolites in *in-vivo*  
310 exercise models. Second, we did not consider sex of the participants, and differences in substrate  
311 oxidation, for example, may affect the observed lactate responses. Third, we do not know  
312 whether the observed effects were due to the duration of the intake. Fourth, maximum oxygen  
313 uptake values are likely indicative of moderately trained individuals and effectiveness in more  
314 trained populations is recommended.

315         In summary, effects of Sujon New Zealand blackcurrant powder for the endurance  
316 athletes during exercise on the lactate curve and resting cardiovascular function may initiate a  
317 new direction in applied sports nutrition research. There is scope to focus on the physiological,  
318 metabolic and performance effects of New Zealand blackcurrant in different exercise modalities.  
319 Intake of Sujon New Zealand blackcurrant powder was associated with 1) a downward and  
320 rightward shift of the lactate curve during cycling over a wide range of exercise intensities, 2)  
321 lower plasma lactate at aerobic capacity suggesting increased lactate clearance or altered

322 substrate oxidation, 3) improved cardiovascular function at rest. These findings may have  
323 implications for training practice, aerobic performance and recovery of endurance athletes.

324

325 Acknowledgement

326 Funding for this study was provided by the University of Chichester, Health Currency Ltd  
327 (United Kingdom) and Gibb Holdings (Nelson) Limited (New Zealand).

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330 References

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332 Bailey, S.J., Winyard, P., Vanhatalo, A. Blackwell, J.R., Dimenna, F.J., Wilkerson, D.P., Tarr, J.,  
333 Benjamin, N., & Jones A.M. (2009). Dietary nitrate supplementation reduces the O<sub>2</sub> cost of low-  
334 intensity exercise and enhances tolerance to high-intensity exercise in humans. *Journal of*  
335 *Applied Physiology (1985)*, 107, 1144-1155. doi: 10.1152/jappphysiol.00722.2009.

336 Bentley, D.J., Newell, J., & Bishop, D. (2007). Incremental exercise test design and analysis:  
337 implications for performance diagnostics in endurance athletes. *Sports Medicine*, 37, 575-586.  
338 doi: 10.2165/00007256-200737070-00002.

339 Bishayee, A., Háznagy-Radnai, E., Mbimba, T., Sipos, P., Morazzoni, P., Darvesh, A.S., Bhatia,  
340 D., & Hohmann, J. (2010). Anthocyanin-rich black currant extract suppresses the growth of  
341 human hepatocellular carcinoma cells. *Natural Product Communications*, 5, 1613-1618. doi:  
342 21121259.

- 343 Bowtell, J.L., Sumners, D.P., Dyer, A., Fox, P., & Mileva, K.N. (2011). Montmorency cherry  
344 juice reduces muscle damage caused by intensive strength exercise. *Medicine & Science in*  
345 *Sports & Exercise*, 43, 1544-1551. doi: 10.1249/MSS.0b013e31820e5adc.
- 346 Brooks, G.A. (1985). Anaerobic threshold: review of the concept and directions for future  
347 research. *Medicine & Science in Sports & Exercise*, 17, 22-34.
- 348 Cassidy, A., Mukamal, K.J., Liu, L., Franz, M., Eliassen, A.H., & Rimm, E.B. (2013). High  
349 anthocyanin intake is associated with a reduced risk of myocardial infarction in young and  
350 middle-aged women. *Circulation*, 127, 188-196. doi:  
351 10.1161/CIRCULATIONAHA.112.122408.
- 352 Connolly, D.A., McHugh, M.P., Padilla-Zakour, O.I., Carlson, L., & Sayers, S.P. (2006).  
353 Efficacy of a tart cherry juice blend in preventing the symptoms of muscle damage. *British*  
354 *Journal of Sports Medicine*, 40, 679-683. doi: 10.1136/bjsm.2005.025429.
- 355 De la Cruz, A.A., Hilbert, G., Mengin, V. Rivière, C., Ollat, N., Vitrac, C., Bordenave,  
356 L., Decroocq, S., Delaunay, J.C., Mérillon, J.M., Monti, J.P., Gomès, E., & Richard, T. (2013).  
357 Anthocyanin phytochemical profiles and anti-oxidant activities of *Vitis candicans* and *Vitis*  
358 *doaniana*. *Phytochemical Analysis*, 24, 446-452. doi: 10.1002/pca.2447.
- 359 Edirisinghe, I., Banaszewski, K., Cappozzo, J., McCarthy, D., & Burton-Freeman, B.M. (2011).  
360 Effect of black currant anthocyanins on the activation of endothelial nitric oxide synthase  
361 (eNOS) in vitro in human endothelial cells. *Journal of Agricultural and Food Chemistry*, 59,  
362 8616-8624. doi: 10.1021/jf201116y.
- 363 Emhoff, C.A., Messonnier, L.A., Horning, M.A., Fattor, J.A., Carlson, T.J., & Brooks, G.A.  
364 (2013). Direct and indirect lactate oxidation in trained and untrained men. *Journal of Applied*  
365 *Physiology* (1985), 115, 829-838. doi: 10.1152/jappphysiol.00538.2013.



- 366 Evertsen, F., Medbø, J.I., & Bonen, A. (2001). Effect of training intensity on muscle lactate  
367 transporters and lactate threshold of cross-country skiers. *Acta Physiologica Scandinavica*, *173*,  
368 195-205. doi: 10.1046/j.1365-201X.2001.00871.x
- 369 Faude, O., Kindermann, W., & Meyer, T. (2009). Lactate threshold concepts: how valid are  
370 they? *Sports Medicine*, *39*, 469-490. doi: 10.2165/00007256-200939060-00003.
- 371 Gladden, L.B. (2008). 200th anniversary of lactate research in muscle. *Exercise and Sport*  
372 *Sciences Reviews*, *36*, 109-115. doi: 10.1097/JES.0b013e31817c0038.
- 373 González-Haro, C., Galilea, P.A., Drobnic, F., & Escanero, J.F. (2007). Validation of a field test  
374 to determine the maximal aerobic power in triathletes and endurance cyclists. *British Journal of*  
375 *Sports Medicine*, *41*, 174-179. doi: 10.1136/bjism.2006.031310.
- 376 Haramizu, S., Ota, N., Hase, T., & Murase, T. (2011). Catechins attenuate eccentric exercise-  
377 induced inflammation and loss of force production in muscle in senescence-accelerated mice.  
378 *Journal of Applied Physiology (1985)*, *111*, 1654-1663. doi: 10.1152/jappphysiol.01434.2010.
- 379 Howatson, G., McHugh, M.P., Hill, J.A., Brouner, J., Jewell, A.P., van Someren, K.A., Shave,  
380 R.E., & Howatson, S.A. (2010). Influence of tart cherry juice on indices of recovery following  
381 marathon running. *Scandinavian Journal of Medicine & Science in Sports*, *20*, 843-852. doi:  
382 10.1111/j.1600-0838.2009.01005.x.
- 383 Jennings, A., Welch, A.A., Fairweather-Tait, S.J., Kay, C., Minihane, A.M., Chowienczyk, P.,  
384 Jiang, B., Cecelja, M., Spector, T., Macgregor, A., & Cassidy, A. (2012). Higher anthocyanin  
385 intake is associated with lower arterial stiffness and central blood pressure in women. *The*  
386 *American Journal of Clinical Nutrition*, *96*, 781-788. doi: 10.3945/ajcn.112.042036.
- 387 Jin, Y., Alimbetov, D., George, T., Gordon, M.H., & Lovegrove, J.A. A randomised trial to

388 investigate the effects of acute consumption of a blackcurrant juice drink on markers of vascular  
389 reactivity and bioavailability of anthocyanins in human subjects. (2011). *European Journal of*  
390 *Clinical Nutrition*, 65, 849-856. doi: 10.1038/ejcn.2011.55.

391 Jordan, T., Lukaszuk, J., Misic, M., & Umoren, J. (2010). Effect of beta-alanine supplementation  
392 on the onset of blood lactate accumulation (OBLA) during treadmill running: Pre/post 2  
393 treatment experimental design. *Journal of the International Society of Sports Nutrition*, 7, 20.  
394 doi: 10.1186/1550-2783-7-20.

395 Kamonpatana, K., Failla, M.L., Kumar, P.S., & Giusti, M.M. (2014). Anthocyanin structure  
396 determines susceptibility to microbial degradation and bioavailability to the buccal mucosa.  
397 *Journal of Agricultural and Food Chemistry*, 62, 6903-6910. doi: 10.1021/jf405180k.

398 Kamonpatana, K., Giusti, M.M., Chitchumroonchokchai, C., MorenoCruz, M., Riedl, K.M.,  
399 Kumar, P., & Failla, M.L. (2012). Susceptibility of anthocyanins to ex vivo degradation in  
400 human saliva. *Food Chemistry*, 135, 738-747. doi: 10.1016/j.foodchem.2012.04.110.

401 Lyall, K.A., Hurst, S.M., Cooney, J., Jensen, D., Lo, K., Hurst, R.D., & Stevenson, L.M. (2009).  
402 Short-term blackcurrant extract consumption modulates exercise-induced oxidative  
403 stress and lipopolysaccharide-stimulated inflammatory responses. *American Journal of*  
404 *Physiology Regul Integr Comp Physiol*, 297, R70-81. doi: 10.1152/ajpregu.90740.2008.

405 Mattila, P.H., Hellström, J., McDougall, G., Dobson, G., Pihlava, J.M., Tiirikka, T.,  
406 Stewart, D., & Karjalainen, R. (2011). Polyphenol and vitamin C contents in European  
407 commercial blackcurrant juice products. *Food Chemistry*, 127, 1216-1223. doi:  
408 10.1016/j.foodchem.2011.01.129.

409 Matsumoto, H., Takenami, E., Iwasaki-Kurashige, K., Osada, T., Katsumura, T., & Hamaoka, T.  
410 (2005). Effects of blackcurrant anthocyanin intake on peripheral muscle circulation during typing

- 411 work in humans. *European Journal Applied Physiology*, 94, 36-45. doi: 10.1007/s00421-004-  
412 1279-y.
- 413 McAnulty, L.S., Miller, L.E., Hosick, P.A., Utter, A.C., Quindry, J.C., & McAnulty, S.R. (2013).  
414 Effect of resveratrol and quercetin supplementation on redox status and inflammation after  
415 exercise. *Applied Physiology, Nutrition, and Metabolism*, 38, 760-765. doi: 10.1139/apnm-2012-  
416 0455.
- 417 McLeay, Y., Barnes, M.J., Mundel, T., Hurst, S.M., Hurst, R.D., Stannard, S.R. (2012). Effect of  
418 New Zealand blueberry consumption on recovery from eccentric exercise-induced muscle  
419 damage. *Journal of the International Society of Sports Nutrition*, 9, 19. doi: 10.1186/1550-2783-  
420 9-19.
- 421 Mendes, A., Desgranges, C., Chèze, C., Vercauteren, J., & Freslon, J.L. (2003). Vasorelaxant  
422 effects of grape polyphenols in rat isolated aorta. Possible involvement of a purinergic pathway.  
423 *Fundamental & Clinical Pharmacology*, 17, 673-681. DOI: 10.1046/j.1472-8206.2003.00198.x.
- 424 Nakamura, Y., Matsumoto, H., & Todoki, K. (2002). Endothelium-dependent vasorelaxation  
425 induced by black currant concentrate in rat thoracic aorta. *The Japanese Journal of*  
426 *Pharmacology*, 89, 29-35. doi: 10.1254/jjp.89.29.
- 427 Newell, J., Higgins, D., Madden, N., Cruickshank, J., Einbeck, J., McMillan, K., & McDonald,  
428 R. (2007). Software for calculating blood lactate endurance markers. *Journal of Sports Sciences*,  
429 25, 1403-1409. doi: 10.1080/02640410601128922.
- 430 Sugawara, J., Tanabe, T., Miyachi, M., Yamamoto, K., Takahashi, K., Iemitsu, M., Otsuki, T.,  
431 Homma, S., Maeda, S., Ajisaka, R., & Matsuda, M. (2003). Non-invasive assessment of cardiac  
432 output during exercise in healthy young humans: comparison between Modelflow method and

- 433 Doppler echocardiography method. *Acta Physiologica Scandinavica*, 179, 361-366. doi:  
434 10.1046/j.0001-6772.2003.01211.x
- 435 Vukovich, M.D., & Dreifort, G.D. (2001). Effect of beta-hydroxy beta-methylbutyrate on the  
436 onset of blood lactate accumulation and V(O)<sub>2</sub> peak in endurance-trained cyclists. *The Journal*  
437 *of Strength and Conditioning Research*, 15, 491-497.
- 438 Wedick, N.M., Pan, A., Cassidy, A., Rimm, E.B., Sampson, L., Rosner, B., Willett, W., Hu,  
439 F.B., Sun, Q., & van Dam, R.M. (2012). Dietary flavonoid intakes and risk of type 2 diabetes in  
440 US men and women. *American Journal of Clinical Nutrition*, 95, 925-933. doi:  
441 10.3945/ajcn.111.028894.
- 442 Xu, J.W., Ikeda, K., & Yamori Y. (2004). Cyanidin-3-glucoside regulates phosphorylation of  
443 endothelial nitric oxide synthase. *FEBS Letters*, 574, 176-180. doi:  
444 <http://dx.doi.org/10.1016/j.febslet.2004.08.027>
- 445 Zembron-Lacny, A., Gajewski, M., Naczek, M., & Siatkowski, I. (2013). Effect of shiitake  
446 (*Lentinus edodes*) extract on antioxidant and inflammatory response to prolonged eccentric  
447 exercise. *Journal of Physiology and Pharmacology*, 64, 249-254.
- 448 Zhu, Y., Ling, W., Guo, H., Song, F., Ye, Q., Zou, T., Li, D., Zhang, Y., Li, G., Xiao, Y., Liu,  
449 F., Li, Z., Shi, Z., & Yang, Y. (2013). Anti-inflammatory effect of purified dietary anthocyanin  
450 in adults with hypercholesterolemia: a randomized controlled trial. *Nutrition, Metabolism and*  
451 *Cardiovascular Diseases*, 23, 843-849. doi: 10.1016/j.numecd.2012.06.005.
- 452 Zhu, Y., Xia, M., Yang, Y., Liu, F., Li, Z., Hao, Y., Mi, M., Jin, T., & Ling, W. (2011). Purified  
453 anthocyanin supplementation improves endothelial function via NO-cGMP activation in  
454 hypercholesterolemic individuals. *Clinical Chemistry*, 57, 1524-1533. doi:  
455 10.1373/clinchem.2011.167361.

456 Ziberna, L., Lunder, M., Tramer, F., Drevenšek, G., & Passamonti, S. (2013). The endothelial  
457 plasma membrane transporter bilitranslocase mediates rat aortic vasodilation induced by  
458 anthocyanins. *Nutrition, Metabolism and Cardiovascular Diseases*, 23, 68-74. doi:  
459 10.1016/j.numecd.2011.02.005.

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461 Figure legends

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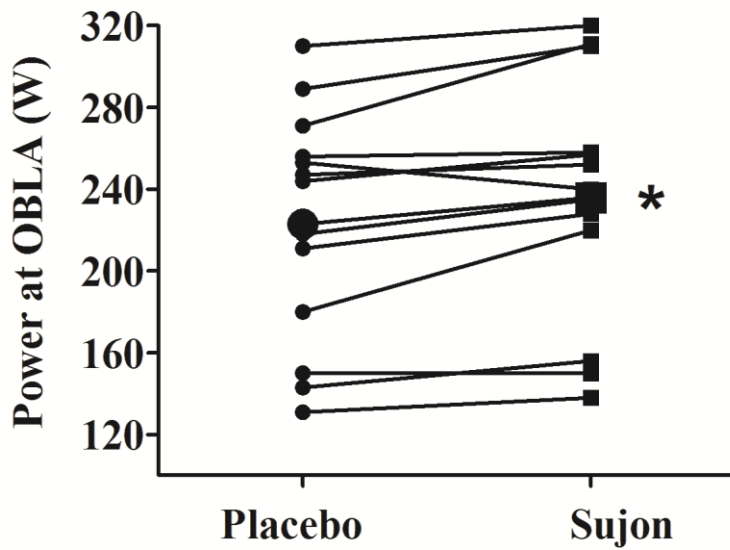
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482 Fig.1. Power values of participants at a lactate value of  $4 \text{ mmol}\cdot\text{L}^{-1}$  (OBLA) for placebo and after  
483 7 days of Sujon New Zealand blackcurrant powder intake. Large symbols indicate mean values.

484 \*, difference between placebo and Sujon New Zealand blackcurrant powder.

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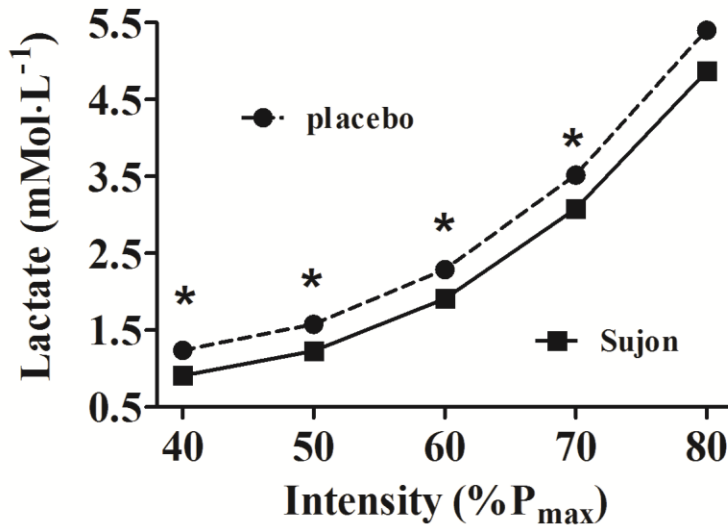
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497 Fig. 2. Relationship between cycling intensity and lactate. Intensity was expressed as a  
498 percentage of maximum power (%P<sub>max</sub>). \*, difference between placebo and Sujon New Zealand  
499 blackcurrant powder. Values are mean±SD.

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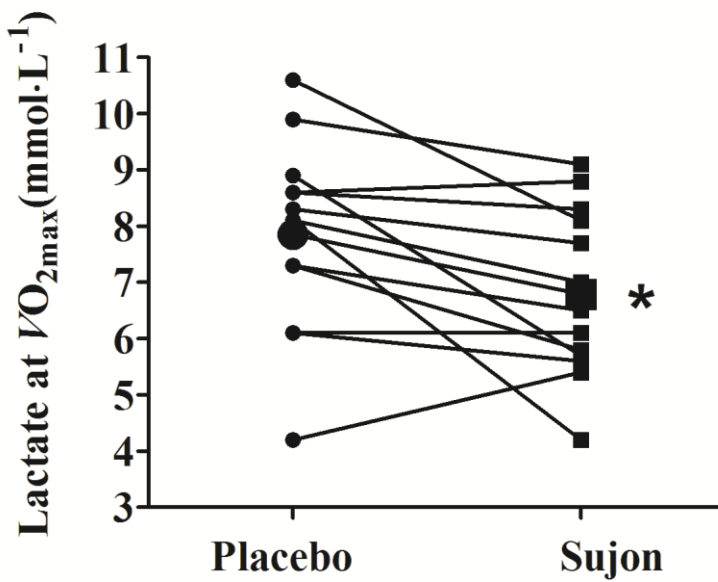
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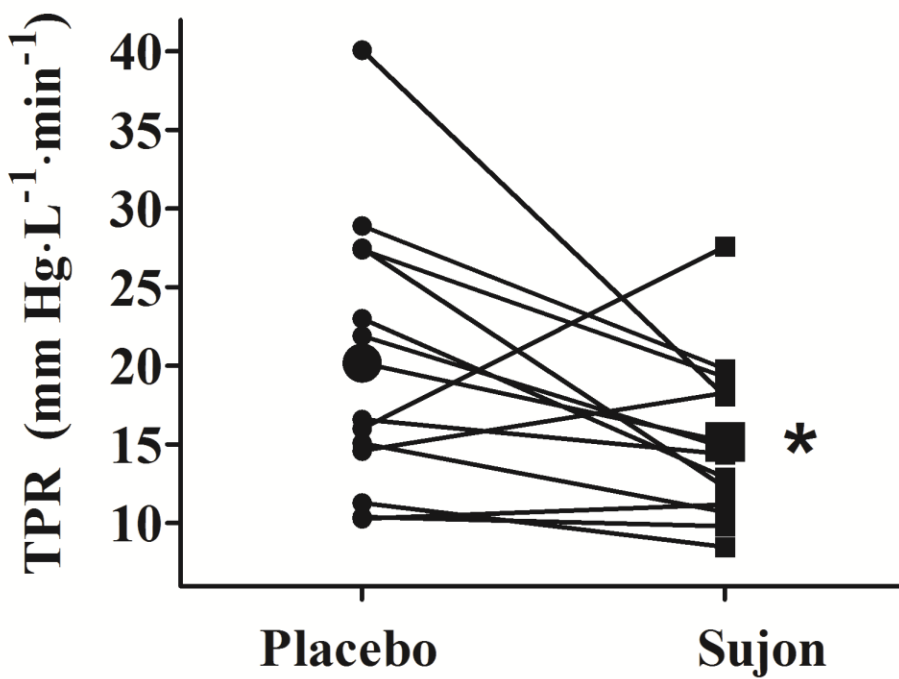
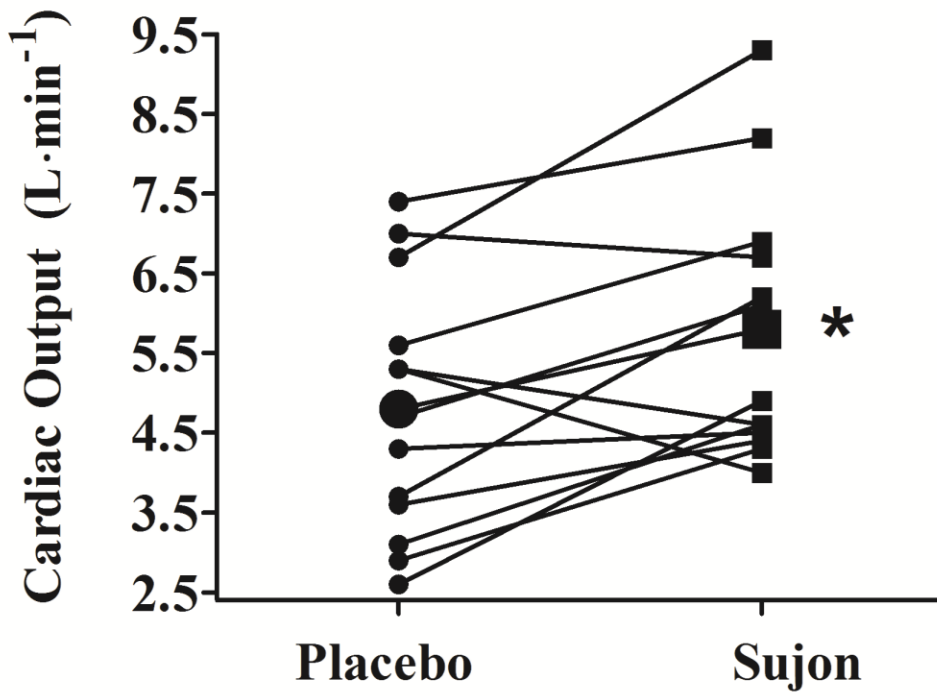


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Fig. 3. Lactate values of participants 3 min after completion of the maximum oxygen uptake protocol. Large symbols indicate mean values. Mean lactate values were lower after 7 days intake of Sujon New Zealand blackcurrant powder. \*, difference between placebo and Sujon New Zealand blackcurrant powder.







526 Fig. 4. Resting cardiac output (A) and resting total peripheral resistance (TPR) (B) of  
527 participants. Large symbols indicate mean values. Mean values for cardiac output and TPR were  
528 higher and lower, respectively, after 7 days intake of Sujon New Zealand blackcurrant powder. \*,  
529 difference between placebo and Sujon New Zealand blackcurrant powder.

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**Table 1.** Cardiovascular responses at during cycling at 40%, 50%, 60%, 70% and 80%  $\dot{V}O_{2\max}$ .

	40%		50%		60%		70%		80%	
	P	N	PB	N	PB	N	PB	N	PB	N
	BO	ZBC	O	ZBC	O	ZBC	O	ZBC	O	ZBC
<b>DBP (mm Hg)</b>	83±15	88±18	84±16	89±19	86±15	92±22	91±14	93±18	97±14	101±19
<b>SPB (mm Hg)</b>	185±31	195±33	188±33	192±40	196±35	203±42	202±35	201±39	215±33	218±35
<b>MAP (mm Hg)</b>	110±20	118±21	114±19	119±23	116±17	122±25	120±16	123±21	129±17	133±20
<b>HR (b·min<sup>-1</sup>)</b>	99±9	100±10	110±8	110±8	124±10	124±10	138±13	139±13	152±13	155±11
<b>SV (mL·min<sup>-1</sup>)</b>	101±18	95±25	96±20	92±25	94±20	93±24	91±18	93±29	88±18	87±28
<b>CO (L·min<sup>-1</sup>)</b>	9.9±1.9	9.6±2.4	10.6±2.4	10.1±2.7	11.7±2.7	11.5±2.9	12.6±2.5	12.9±4.2	13.3±2.6	13.6±4.4
<b>TPR (mmHg·L<sup>-1</sup>·min<sup>-1</sup>)</b>	11.8±4.5	13.5±5.9	11.7±4.9	13.1±6.3	10.7±4.2	11.8±6.2	10.1±3.1	10.7±4.7	10.3±3.3	11.3±5.5

534 PBO, placebo; NZ BC, Sujon New Zealand blackcurrant; DPB, diastolic blood pressure; SBP, systolic blood pressure; MAP, mean arterial  
535 pressure; HR, heart rate; SV, stroke volume; CO, cardiac output; TPR, total peripheral resistance. Data of ten subjects. **There were no differences**  
536 **between placebo and Sujon New Zealand blackcurrant for a cardiovascular response at each intensity.** Values are mean±SD.

