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1 Title of Article: Cardiovascular Function during Supine Rest in Endurance Trained
2 Males with New Zealand Blackcurrant: A Dose-Response Study

3

4 Authors: Matthew David Cook, Stephen David Myers, Mandy Lucinda
5 Gault, Victoria Charlotte Edwards, Mark Elisabeth Theodorus
6 Willems

7

8 Affiliation: University of Chichester
9 Department of Sport & Exercise Sciences
10 College Lane
11 Chichester, PO19 6PE
12 United Kingdom

13

14 Corresponding author: Mark Willems
15 University of Chichester
16 Department of Sport & Exercise Sciences
17 College Lane
18 Chichester, PO19 6PE
19 United Kingdom

20

21 Phone: +44 (0)1243 816468

22 Email: m.willems@chi.ac.uk

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31 **ABSTRACT**

32 **Purpose** Blackcurrant contains anthocyanins that could alter cardiovascular function and reduce
33 cardiovascular disease risk. We examined dose responses of New Zealand blackcurrant (NZBC) extract
34 on cardiovascular function during supine rest.

35 **Methods** Fifteen endurance trained male cyclists (age: 38 ± 12 years, height: 178 ± 5 cm, body mass:
36 76 ± 10 kg, $\dot{V}O_{2\max}$: 56 ± 8 mL \cdot kg $^{-1}\cdot$ min $^{-1}$, mean \pm SD) were randomly assigned using a counterbalanced
37 Latin square design to complete four conditions, a control of no NZBC, or one of three doses (300, 600
38 or 900 mg \cdot day $^{-1}$) of NZBC extract (CurraNZTM) for seven-days with a fourteen-day washout.
39 Cardiovascular function (i.e. blood pressure, heart rate, ejection time, cardiac output, stroke volume
40 and total peripheral resistance) during supine rest was examined (Portapres[®] Model 2).

41 **Results** Systolic and diastolic blood pressure, heart rate and ejection time were unchanged by NZBC.
42 A dose effect ($P<0.05$) was observed for cardiac output, stroke volume and total peripheral resistance.
43 A trend for a dose effect was observed for mean arterial blood pressure. Cardiac output increased by
44 0.6 ± 0.6 L \cdot min $^{-1}$ (15%) and 1.0 ± 1.0 L \cdot min $^{-1}$ (28%) and stroke volume by 5 ± 8 mL (7%) and 6 ± 17 mL
45 (18%) between control and 600, and 900 mg \cdot day $^{-1}$, respectively. Total peripheral resistance decreased
46 by 4 ± 3 mmHg \cdot L $^{-1}\cdot$ min $^{-1}$ (20%) and 5 ± 9 mmHg \cdot L $^{-1}\cdot$ min $^{-1}$ (20%) for 600, and 900 mg \cdot day $^{-1}$.

47 **Conclusion** Seven-days intake of New Zealand blackcurrant extract demonstrated dose-dependent
48 changes on some cardiovascular parameters during supine rest in endurance-trained male cyclists.

49

50 **Keywords:** Cardiovascular function; New Zealand blackcurrant; anthocyanins; sports nutrition;
51 polyphenols.

52

53 **Abbreviations:**

54	FMD	flow-mediated dilation
55	NADPH	Nicotinamide-adenine dinucleotide phosphate
56	NZBC	New Zealand blackcurrant
57	$\dot{V}O_{2\max}$	Maximal rate of oxygen uptake
58	WR _{max}	Maximum work rate

59

60 **INTRODUCTION**

61 Blackcurrant (*Ribes nigrum*) is a rich source of flavonoids, especially the anthocyanins delphinidin-3-
62 rutinoid, delphinidin-3-glucoside, cyanidin-3-rutinoid and cyanidin-3-glucoside (Kähkönen et al.
63 2003). In animal studies, anthocyanins induced vasodilation and relaxation in thoracic aortic rings in
64 male Wistar rats, and prevented loss of endothelium-dependent relaxation by exposure to exogenous
65 reactive oxygen species in porcine arteries (Bell and Gochenaur 2006). Such observations in humans
66 may, in the long term, reduce cardiovascular risk factors. Indeed, numerous epidemiological studies
67 indicate that consumption of foods high in flavonoids can reduce the risk of cardiovascular disease
68 (Huxley and Neil 2003; Mink et al. 2007).

69 In *in vitro* animal studies, physiological responses have shown dose-response effects to
70 anthocyanins. For example, blackcurrant concentrate induced dose-dependent relaxation on
71 norepinephrine contracted rat aorta (Nakamura et al. 2002) and incubation of bovine arterial cells with
72 cyanidin-3-glucoside increased endothelial nitric oxide synthase (eNOS) expression in a dose-
73 dependent manner (Xu et al. 2004a). However, caution is required to generalise findings from *in vitro*
74 observations with anthocyanins on arteries and myocardium to *in vivo* human conditions due to the low
75 bioavailability of anthocyanins and possible additional cardiovascular effects by the anthocyanin
76 metabolites. Increases in circulating anthocyanin metabolites were linked with a dose-dependent
77 increase in flow-mediated dilation (FMD) up to 310 mg of blueberry anthocyanins with higher doses
78 having no further increases (Rodriguez-Mateos et al. 2013).

79 However, studies that highlighted a dose-response effect of intake of berry anthocyanins on
80 cardiovascular parameters were executed in healthy untrained subjects (Rodriguez-Mateos et al. 2013,
81 2016). We observed in endurance trained athletes that a daily intake of New Zealand blackcurrant
82 powder for seven days increased stroke volume and cardiac output by 25% and 26%, respectively, and
83 total peripheral resistance was decreased by 16% with no changes in systolic, diastolic or mean arterial
84 blood pressure during supine rest (Willems et al. 2015). This observation was with a daily intake of
85 138.6 mg·day⁻¹ of blackcurrant anthocyanins and it is not known whether there is dose-dependent effect
86 on cardiovascular function during supine rest. The dose-dependent cardiovascular responses to berry
87 anthocyanin intake are unknown for those regularly undertaking endurance training, which possess
88 already cardiovascular adaptations by the endurance training (for a review see Hellsten and Nyberg
89 2015). It is possible that an endurance trained cardiovascular system may not clearly respond to dose
90 effects of anthocyanin intake. We therefore hypothesized that there would be no dose-response effects

91 of a rich berry anthocyanin-containing extract on cardiovascular function during supine rest in trained
92 male cyclists. The aim of the present study was to examine the dose-response effects of New Zealand
93 blackcurrant extract on cardiovascular function at supine rest in trained male cyclists.

94

95 **METHODS**

96 **Participants**

97 Fifteen endurance trained men (age: 38 ± 12 years, height: 178 ± 5 cm, body mass: 76 ± 10 kg, $\dot{V}O_{2max}$:
98 57 ± 8 mL·kg⁻¹·min⁻¹, WR_{max} : 378 ± 55 W) provided written informed consent to participate in the study.
99 Participants were recruited from local cycling clubs with a history of cycling participation of greater
100 than 3 years and were not involved in a structured training programme for the study duration, but
101 typically performed cycling exercise for 6 to 10 hours a week. All participants were non-smokers and
102 they were taking no nutritional supplements. The study was approved by the University of Chichester
103 Research Ethics Committee with protocols and procedures conforming to the 2013 Declaration of
104 Helsinki.

105 **Experimental Design**

106 Participants visited the laboratory for 5 visits at the same time of day (8:00am). Before arrival,
107 participants were instructed to abstain from vigorous exercise for 48 hours, alcohol for 24 hours and
108 caffeine-containing products on the day of testing. Before commencing data collection on that visit,
109 participants verbally acknowledged compliance to the experimental requirements. During the first visit,
110 stature (Seca 213, Seca, Birmingham, UK), body mass (Kern ITB, Kern, Balingen, Germany) and body
111 fat (Tanita BC418 Segmental Body Composition analyzer, Tanita, Illinois, USA) were measured.
112 Subsequently, participants completed an incremental intensity maximal cycling test to volitional
113 exhaustion for calculation of maximal oxygen uptake ($\dot{V}O_{2max}$) and maximum work rate (WR_{max} ; the
114 last complete work rate, plus the fraction of time spent in the final non-completed work rate multiplied
115 by the work rate) on an electronically controlled cycle ergometer (SRM ergometer, SRM International,
116 Jülich Germany).

117 Participants were assigned, in a randomised, counterbalanced Latin-square design, to three NZBC
118 doses (i.e. 1, 2 or 3 capsules a day) for seven-days and one control condition of no dose. The 300 mg
119 active cassis capsules contained 105 mg of anthocyanins, consisting of 35-50% delphinidin-3-
120 rutinoside, 5-20% delphinidin-3-glucoside, 30-45% cyanidin-3-rutinoside, 3-10% cyanidin-3-glucoside

121 (CurraNZ™, Health Currancy Ltd, Surrey, UK). Participants were instructed to take the capsules, with
122 breakfast (one capsule per day, 300 mg·day⁻¹ condition), 12 hours apart (two capsules per day, 600
123 mg·day⁻¹ condition) and evenly spaced through the day (three capsules per day, 900 mg·day⁻¹
124 condition). Optimal dosing duration of NZBC extract is not known. However, previous studies on the
125 effectiveness of berry juice intake in humans also used multiple days of intake (Connolly et al. 2006;
126 Howatson et al. 2010).

127 On the final day of supplementation, participants reported to the laboratory, two hours post-prandial of
128 a standard breakfast (i.e. one slice of buttered bread or toast ~840 kJ, ~30 g carbohydrate, ~6 g protein
129 and ~7 g fat) and all the capsules required for that condition. Between laboratory visits, there was a
130 fourteen-day washout period. An anthocyanin intake for one month similar to highest dose in the
131 present study returned biochemical and biomarkers of antioxidant status to baseline of after a fifteen-
132 day washout (Alvarez-Suarez et al. 2014). The NZBC capsules were independently analysed and
133 confirmed the ingredients present with an absence of caffeine. Participants then rested for 5 minutes in
134 a supine position before beat-to-beat blood pressure (Portapres® Model 2, Finapres Medical Systems
135 BV, Amsterdam, The Netherlands) was recorded for 20-minutes during supine rest (see below).
136 Cardiovascular responses in rest are affected by posture position (Nishiyasu et al. 1998).

137 **Anthocyanin Consumption, Physical Activity and Dietary Standardization**

138 Participants completed a food frequency questionnaire that listed the amount and frequency of
139 anthocyanin containing foods and drinks compiled from the Phenol Explorer database (Neveu et al.
140 2010). Daily anthocyanin intake was calculated as the sum of consumption frequency of each food
141 multiplied by the anthocyanin content for the portion size. Daily intake of anthocyanins was calculated
142 to be 67±47 mg·day⁻¹.

143 Participants were instructed to keep their weekly exercise schedule as consistent as possible. All
144 participants recorded their dietary intake and exercise on a written diary 48 hours prior to the first
145 experimental condition (i.e. visit 2) and were then told to replicate this for all subsequent experimental
146 visits (i.e. visits 3, 4, 5) using the first diary as a guide, while recording on a new diary their dietary
147 intake and exercise for that visit. Food diaries were analysed using Nutritics (Nutritics LTD, Dublin,
148 Ireland) for carbohydrate, fat and protein intake and total energy intake (kJ).

149 There were no differences ($P>0.05$) in absolute or relative per kilogram of body mass values for
150 carbohydrate, fat, protein, or total energy for 48 hours prior to each experimental visit (Table 1).

151 Analysis of the food diaries identified that all participants reported 100% adherence to the dietary
152 instructions 48 hours prior to each visit.

153 **Maximal Rate of Oxygen Uptake**

154 $\dot{V}O_{2\max}$ and WR_{\max} were determined with an incremental intensity cycling test to volitional exhaustion.

155 The test began at 50 W for 4 minutes and subsequently increased by 30 W each minute with
156 participants instructed to keep pedal cadence between 70 and 90 $\text{rev}\cdot\text{min}^{-1}$. Expired air samples were
157 collected using the Douglas bag technique with separate air samples collected for a minimum of 3-
158 minutes before participants reached volitional exhaustion. Expired air was analysed with a three-
159 pointed calibrated gas analyser (Series 1400, Servomex, Crowborough, UK), and volume measured
160 (Harvard Apparatus Ltd., Edenbridge, UK). Gas volumes were calculated using Haldane
161 transformation and standardisation to STPD conditions, with consideration of inspired fraction of
162 oxygen and carbon dioxide measured within the laboratory during the protocol. $\dot{V}O_{2\max}$ and WR_{\max} were
163 measured in visit 1.

164 **Cardiovascular Function Measurements**

165 Cardiovascular responses were recorded using a beat-to-beat blood pressure monitoring system during
166 20 minutes of rest in a supine position using the arterial volume clamp method (Penaz 1973). The
167 Portapres® is a beat-to-beat finger blood pressure analyser that allows the non-invasive continuous
168 measurement of haemodynamic parameters. The cardiac output calculated by the Portapres has shown
169 to be significantly correlated ($r=0.87$, $P<0.01$) with cardiac output measurements by ultrasound
170 Doppler from rest up to 130% of the ventilatory threshold during semi-supine cycling (Sugawara et al.
171 2003). The finger cuff was positioned around the same finger of the left hand. Cardiovascular measures
172 were averaged over 10 consecutive beats, with the lowest systolic blood pressure and associated
173 measures recorded. Systolic blood pressure, diastolic blood pressure, mean arterial blood pressure,
174 heart rate, ejection time, cardiac output, stroke volume and total peripheral resistance were recorded
175 (Beatscope 1.1a., Finapres Medical Systems BV, Amsterdam, The Netherlands).

176 **Statistical Analysis**

177 An a-priori power analysis indicated a sample size of 15 would allow a detection of a 26% increase in
178 cardiac output with a high statistical power ($1 - \beta = 0.95$; $0.05 = \alpha$ level). Statistical analyses were
179 completed using SPSS 20.0 (SPSS, Chicago, USA). Differences between the dosing conditions (0 vs.
180 300 vs. 600 vs. 900 $\text{mg}\cdot\text{day}^{-1}$) were analysed with a one-way within subjects analysis of variance

181 (ANOVA) with between dose condition difference examined with a paired samples *t*-test. Mauchley's
182 Test of Sphericity was conducted to test for homogeneity of data and where violations were present
183 Greenhouse-Geisser adjustments were made. To determine the effect size of responses, Cohen's *d* were
184 calculated with Cohen (1988) describing an effect size of <0.2 as trivial, 0.2-0.39 as a small, 0.4-0.69
185 as a moderate and ≥ 0.7 as a large magnitude of change. Statistical significance was accepted at $P < 0.05$.
186 Interpretation of $0.05 \geq P \leq 0.1$ as a trend was according to guidelines by Curran-Everett and Benos
187 (2004).

188

189 RESULTS

190 There were no differences between the dosing conditions for systolic blood pressure ($P=0.35$), diastolic
191 blood pressure ($P=0.60$), heart rate ($P=0.56$) and ejection time ($P=0.52$) (Figures 1 a, b, c and d,
192 respectively). There was a dose effect of NZBC on mean arterial pressure ($P=0.023$), cardiac output
193 ($P < 0.001$), stroke volume ($P=0.014$) and total peripheral resistance ($P=0.012$) (Figures 1 e, f, g and h,
194 respectively).

195 Mean arterial pressure (Fig. 1e) exhibited a decrease of 7 ± 9 mmHg (8%, 11 of 15 participants
196 decreased, $d=0.76$) between 0 and 600 mg·day⁻¹ and 5 ± 7 mmHg (6%, 14 of 15 participants decreased,
197 $d=0.69$) between 300 and 900 mg·day⁻¹ ($P < 0.05$). There was a trend for a lower mean arterial pressure
198 of 5 ± 11 mmHg (6%) ($P=0.1$) between 0 and 900 mg·day⁻¹ and 7 ± 12 mmHg (7%) ($P=0.05$) between
199 300 and 600 mg·day⁻¹. NZBC increased cardiac output by 0.6 ± 0.6 L·min⁻¹ (15%, 14 of 15 participants
200 increased, $d=0.93$), 1.0 ± 1.0 L·min⁻¹ (28%, 11 of 15 participants increased, $d=0.94$) and 0.6 ± 0.9 L·min⁻¹
201 (15%, 13 of 15 participants increased, $d=0.67$) between 0 and 600 mg·day⁻¹, 0 and 900 mg·day⁻¹ and
202 300 and 900 mg·day⁻¹ (all $P < 0.05$), respectively (Fig. 1f). Between 0 and 600 mg·day⁻¹ and 0 and 900
203 mg·day⁻¹, stroke volume (Fig. 1g) increased by 5 ± 8 mL (7%, 13 of 15 participants increased, $d=0.70$)
204 and 6 ± 17 mL (18%, 13 of 15 participants increased, $d=0.95$), respectively. For total peripheral
205 resistance (Fig. 1h), a decrease of 4 ± 3 mmHg·L⁻¹·min⁻¹ (20%, 13 of 15 participants decreased, $d=1.29$),
206 5 ± 9 mmHg·L⁻¹·min⁻¹ (20%, 13 of 15 participants decreased, $d=0.60$) and 3 ± 4 mmHg·L⁻¹·min⁻¹ (15%,
207 11 of 15 participants, $d=0.78$) was observed between 0 and 600 mg·day⁻¹, 0 and 900 mg·day⁻¹ and 300
208 and 900 mg·day⁻¹ ($P < 0.05$), respectively.

209

210 DISCUSSION

211 This is the first study to examine the dose-response effects of NZBC extract on cardiovascular function
212 during supine rest in trained male cyclists. The principle finding from the present study was that NZBC
213 extract increased cardiac output and stroke volume, and decreased total peripheral resistance in a dose-
214 dependent manner in endurance trained male cyclists, with changes having moderate and large effect
215 sizes. There was a trend for a dose effect for mean arterial blood pressure.

216 Willems et al. (2015) also observed no changes in systolic or diastolic blood pressure and heart rate
217 following seven-days intake of NZBC powder in trained male and female triathletes. However,
218 increases in cardiac output by 25%, stroke volume by 26%, and a decrease in total peripheral resistance
219 by 16% were observed (Willems et al. 2015). The present study observed similar group mean
220 increases, but following a dose almost three times that of Willems et al (2015) (~ 139 vs ~ 315 $\text{mg}\cdot\text{day}^{-1}$
221 anthocyanin). This may have resulted from the different way in which NZBC was delivered. Willems
222 et al (2015) used NZBC powder dissolved in water while the present study used capsulated NZBC
223 extract which may affect absorption rate of anthocyanin and also bypasses the potentially degrading
224 properties of saliva (Kamonpatana et al. 2012). Additionally, Willems et al (2015) observed no change
225 in mean arterial pressure, whereas in this study differences between 0 and 600 and 900 $\text{mg}\cdot\text{day}^{-1}$ were
226 observed with large and moderate effect sizes, respectively. This indicates that higher intakes of
227 anthocyanins are associated with reduced mean arterial pressure (Jennings et al. 2012).

228 The dose-dependent cardiovascular function responses during supine rest in endurance trained
229 individuals in the present study support those studies examining the dose-response relationships of
230 anthocyanin on FMD in healthy untrained individuals. For example, Rodriguez-Mateos et al (2013)
231 reported a dose-dependent increase in FMD up to 310 mg anthocyanin, and then a plateau above this
232 dose. The present study observed no significant increases between 600 and 900 $\text{mg}\cdot\text{day}^{-1}$ NZBC (210
233 and 315 $\text{mg}\cdot\text{day}^{-1}$ anthocyanin, respectively) on any cardiovascular parameter, indicating a levelling off
234 in cardiovascular responses during supine rest with a dose of 600 $\text{mg}\cdot\text{day}^{-1}$ NZBC extract. However,
235 the responses above 900 $\text{mg}\cdot\text{day}^{-1}$ NZBC extract are unknown. It is possible, however, that a plateau on
236 cardiovascular function exists in a similar fashion to the results of the study by Rodriguez-Mateos et al
237 (2013), as uptake of higher intakes of NZBC extract may be limited by mechanisms for anthocyanin
238 absorption (Kurilich et al. 2005).

239 Upon ingestion, anthocyanins have poor bioavailability (Czank et al. 2013). Their uptake is affected by
240 gut microflora [for review see Kemperman et al. (2010)], with inter-individual variations in gene

241 content of gut bacterial species of 13% observed (Zhu et al. 2015). Furthermore, George et al (2012)
242 observed that expression of the Glu298Asp single nucleotide polymorphism in the endothelial nitric
243 oxide synthase gene differentially affects the endothelium-dependent vasodilation response to a fruit
244 and vegetable puree drink. Taken together, such factors may explain the inter-individual variation for
245 NZBC extract on cardiovascular function responses during supine rest.

246 Blackcurrant anthocyanins are quickly absorbed and excreted with values reaching maximum plasma
247 concentrations within 2 hours (Matsumoto et al. 2001). Therefore, metabolites of anthocyanins, or
248 synergistic action of metabolites, could lead to the cardiovascular responses during supine rest. In
249 addition, metabolites have been shown to remain within the plasma for 48 hours following intake
250 (Czank et al. 2013). Therefore, a build-up of metabolites over the 7-day supplementation period within
251 the present study and effects of the metabolites may have caused the altered cardiovascular function
252 during supine rest. However, we cannot exclude that the cardiovascular responses during supine rest in
253 the present study may have been caused by acute responses to the anthocyanin intake as measurements
254 were taken 2 hours after intake. In both Willems et al. (2015) and the present study, the last intake
255 across the seven days was taken 2 hours before the recording of cardiovascular function during supine
256 rest. This is supported by observations that increases in FMD have occurred 1-2 hours following an
257 intake of blueberry polyphenols and coincides with a peak in phenolic metabolites such as ferulic acid,
258 isoferulic acid, vanillic acid, 2-hydroxybenzoic acid, benzoic acid and caffeic acid in the plasma
259 (Rodriguez-Mateos et al. 2013), but anthocyanin composition of blueberries differ from blackcurrant
260 with potential consequences for the occurrence of plasma metabolites. Similarly, Kent et al. (2016)
261 observed that a single serving of cherry juice (~207 mg anthocyanins) reduced systolic and diastolic
262 blood pressure and heart rate 2 hours following intake and this coincided with a peak in caffeic acid.
263 Therefore, future studies should examine the acute responses for cardiovascular function during supine
264 rest to NZBC extract intake with measurement of phenolic metabolites. It is possible that these
265 phenolic metabolites maybe responsible for the possible mechanisms for the observed effect in the
266 present study. For example, they have been observed to influence human vascular smooth muscle cell
267 behaviour *in vitro* (Keane et al. 2016a) and may also increase nitric oxide availability, as shown by
268 inhibiting NAPH oxidase (Rodriguez-Mateos et al. 2013) and increasing endothelial nitric oxide
269 synthase expression (Xu et al. 2004b). While these effects upon expression and activity of nitric oxide
270 would potentially result in vascular responses, Keane et al. (2016b) observed plasma nitrite and nitrate

271 (surrogate markers for nitric oxide production) to be unaffected by cherry anthocyanins. Therefore, the
272 effects of anthocyanin metabolites on vascular smooth cell behaviour seems the most likely mechanism
273 for the cardiovascular responses, which lead to a decrease in total peripheral resistance and mean
274 arterial pressure in the present study. Whilst indirect, the decrease in total peripheral resistance also
275 suggests an increased peripheral blood flow during supine rest as changes in arterial diameter influence
276 blood flow (Mayet and Hughes 2003), an observation which has been previously been made following
277 intake of blackcurrant anthocyanins (Matsumoto et al. 2005). However, the combination of decreased
278 total peripheral resistance and mean arterial pressure with increased cardiac output and stroke volume
279 with no change in heart rate and systolic or diastolic blood pressure suggests more complex
280 mechanisms. For example, an elevation of mean arterial pressure can only result from an increase in
281 cardiac output, an increase in total peripheral resistance, or both (Mayet and Hughes 2003). However, a
282 decreased mean arterial pressure and total peripheral resistance as in this study indicates greater venous
283 return resulting in the increased cardiac output from a larger end diastolic filling during the cardiac
284 cycle.

285 **Limitations**

286 For the present study, various limitations should be considered. Firstly, the short time frame of the
287 present study does not indicate benefits for longer-term consumption and cardiovascular health.
288 Secondly, the study population consisted of healthy men who regularly participate in cycling exercise
289 and observations cannot be extended to the general population, and further work is required to identify
290 whether similar cardiovascular responses would occur in women, untrained populations and those with
291 cardiovascular disease conditions. However, future work should examine the potential consequences of
292 increased cardiac output in rest on cardiomyocyte oxygen consumption. Thirdly, the present study
293 supplemented with capsules of NZBC extract. Therefore, these results are limited to this delivery
294 mechanism and it is unknown if similar responses are observed from whole unprocessed blackcurrant
295 intake. Finally, in present study, dietary intake was controlled for 48 hours before each visit, with no
296 differences observed, but the total polyphenol intake was not measured. Therefore, we cannot exclude
297 that the intake of dietary polyphenols including anthocyanins acted synergistically with the NZBC
298 anthocyanin intake in the present study.

299 **Conclusion**

300 In conclusion, New Zealand blackcurrant extract taken in capsules for seven-days increased cardiac
301 output and stroke volume, and decreased mean arterial pressure and total peripheral resistance during
302 supine rest in a dose-dependent manner up to a daily intake of 900 mg·day⁻¹ (315 mg·day⁻¹ anthocyanin)
303 in endurance trained male cyclists. While anthocyanins have been shown to influence cardiovascular
304 responses in diseased and untrained populations, these findings indicate that anthocyanins also alter
305 cardiovascular function during supine rest in endurance trained cyclists in a dose-dependent manner. In
306 a previous study with the lowest dose of New Zealand blackcurrant as used in the present study, we did
307 not observe differences in cardiovascular responses between 40% and 80% of maximum power
308 (Willem et al. 2015). Future work should examine whether higher doses of New Zealand blackcurrant
309 affects the cardiovascular responses during exercise.

310

311 **Acknowledgments**

312 Supply of supplement (CurraNZ™) for this study was obtained from Health Currancy Ltd (United
313 Kingdom).

314

315 **Conflict of Interest**

316 The authors declare no other conflicts of interest.

317

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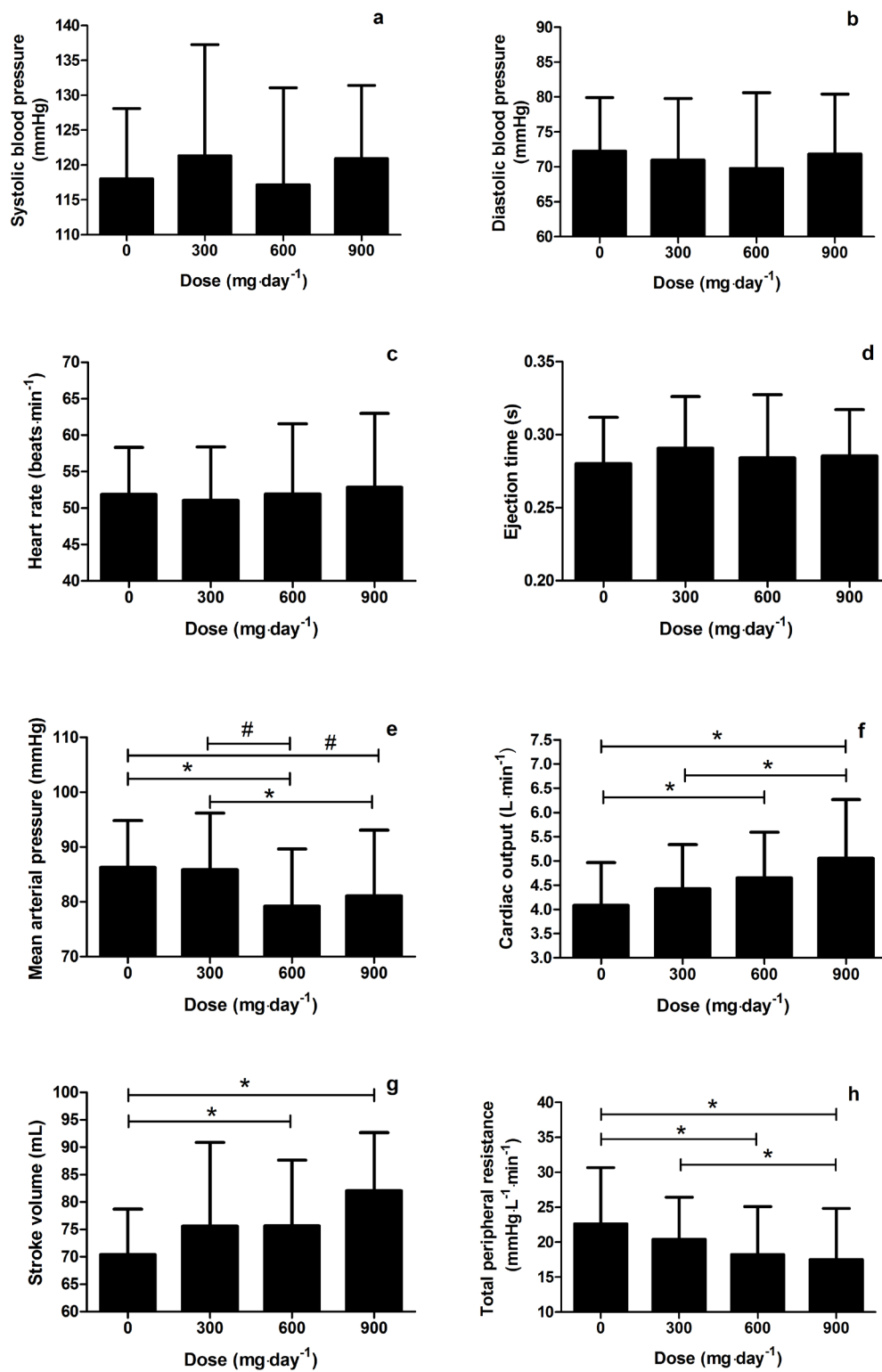
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420 **Fig. 1.** a: Systolic blood pressure, b: Diastolic blood pressure, c: Heart rate, d: Ejection time, e: Mean

421 arterial pressure, f: Cardiac output, g: Stroke volume, h: Total peripheral resistance during supine rest

422 following 0 or 300, 600 and 900 mg·day⁻¹ of New Zealand blackcurrant extract in 15 endurance trained
 423 male cyclists. Data are mean±SD. * indicates difference between doses ($P<0.05$), # indicates a trend
 424 between doses

425

426 Table 1. Dietary intake 48 hours before each visit for each treatment condition.

	0 mg·day ⁻¹	300 mg·day ⁻¹	600 mg·day ⁻¹	900 mg·day ⁻¹
Carbohydrate (g)	494±91	495±90	479±85	490±101
(g·kg body mass ⁻¹)	6.7±1.8	6.7±1.7	6.5±1.6	6.6±1.9
Fats (g)	228±68	228±68	230±65	235±73
(g·kg body mass ⁻¹)	3.1±1.0	3.1±0.9	3.1±0.9	3.1±1.0
Protein (g)	216±59	221±58	217±56	220±60
(g·kg body mass ⁻¹)	2.9±0.9	3±0.9	2.9±0.8	3.0±0.9
Total Energy Intake (kJ)	20654±2950	20804±3080	20724±2805	20709±2835
(kJ·body mass ⁻¹)	279±63	280±59	279±56	278±54

427 Intake of dietary variables for the different NZBC dosing conditions of 0, 300, 600 and 900 mg·day⁻¹.
 428 All values were collected from 48-hour food diaries before each experimental visit. Data reported as
 429 mean ± SD from 15 endurance trained male cyclists.
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