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No effects of different doses of New Zealand blackcurrant extract on cardiovascular responses during rest and submaximal exercise across a week in trained male cyclists

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Running Title: Blackcurrant responses in trained cyclists

Abstract: Supplementation with anthocyanin-rich blackcurrant increases blood flow, cardiac output, and stroke volume at rest. It is not known if cardiovascular responses can be replicated over longer timeframes in fed trained cyclists. In a randomized, double-blind, crossover design, thirteen male trained cyclists (age 39 ± 10 years, $\dot{V}O_{2max}$ 55.3 ± 6.7 $ml \cdot kg^{-1} \cdot min^{-1}$) consumed two doses of New Zealand blackcurrant (NZBC) extract (300 and 600 $mg \cdot day^{-1}$ for one week). Cardiovascular parameters were measured during rest and submaximal cycling (65% $\dot{V}O_{2max}$) on day 1 (D1), D4 and D7. Data were analyzed with a RM ANOVA using dose (PLA vs 300 vs 600 $mg \cdot day^{-1}$) by time point (D1, D4 and D7). Outcomes from PLA were averaged to determine the coefficient of variation (CV) within our experimental model, and 95%CI were examined for differences between PLA and NZBC. There were no differences in cardiovascular responses at rest between conditions and between days. During submaximal exercise, no positive changes were observed on D1 and D4 after consuming NZBC extract. On D7, intake of 600 mg increased stroke volume (3.08 ml, 95%CI: -2.08, 8.26; d=0.16, p=0.21), cardiac output (0.39 $L \cdot min^{-1}$, 95%CI: -1.39, 0.60; d=0.14, p=0.40) (both +2.5%) and lowered total peripheral resistance by 6.5% (-0.46 $mmHg \cdot min \cdot mL^{-1}$, 95%CI: -1.80, 0.89; d=0.18, p=0.46). However, these changes were trivial and fell within the CV of our study design. Therefore, we can conclude that NZBC extract was not effective in enhancing cardiovascular function during rest and submaximal exercise in endurance trained fed cyclists.

Introduction

During endurance exercise, the increment in oxygen consumption is predominantly dictated by the metabolic demand of skeletal muscles. Cardiac output increases to meet the oxygen demand during endurance exercise (Hellsten and Nyberg, 2016). Elite athletes can sustain high intensity workload (85% $\dot{V}$O2max) for several hours, with cardiac output reaching up to 35-40 L·min^{-1} (Ekblom and Hermansen, 1968). Therefore, oxygen delivery at a given workload is paramount to sustain muscle contractions during high intensity endurance exercise.

In the last decade, fruit-derived (e.g. blackcurrant, chokeberry and blueberries) supplements have been examined due to their ability to ameliorate cardiovascular function, reducing oxidative stress and inflammation (e.g. cherry, Bell et al., 2015). Berries are rich in anthocyanins, water soluble molecules that belong to the flavonoid group of polyphenols and responsible for the red, blue and purple colours of plant and fruits (Harborne & Grayer, 1988). A recent meta-analysis showed that acute and chronic consumption of anthocyanins can improve flow mediated dilatation (FMD) (Fairlie-Jones et al., 2017). *In vitro* studies have shown that anthocyanins play a role in vascular health. Anthocyanins enter the endothelial smooth cells (Ziberna et al., 2012) and enhance gene expression of endothelial nitric oxide synthase (eNOS), an enzyme responsible for production of the vasodilator nitric oxide (NO) (Xu et al., 2004). Matsumoto et al., (2005) observed that acute intake of 17 mg·kg^{-1} of blackcurrant concentrate increased forearm blood flow by 1.22 (0.13)-fold, 2 h post ingestion. This response seems to be dose dependent reaching a plateau around intake of 310 mg of anthocyanins (Rodriguez-Mateos et al., 2013). The rise in blood flow coincided with peak plasma anthocyanin (Czank et al., 2013). However, anthocyanins are rapidly metabolized and their products are still present in the plasma up to 48 h (de Ferrars et al., 2014). These second phase metabolites seem to be beneficial to vascular function by increasing NO bioavailability via reduction of nicotinamide adenine nucleotide (NADH) activity (Rodriguez-Mateos et al.,

2013). Therefore, studies of anthocyanin-rich supplements have implemented strategies for short-term intake (e.g. 7 days) based on the premise of the build-up of metabolites in tissues and plasma over time. Willems et al., (2015) showed that consuming ~138 mg of anthocyanins from New Zealand blackcurrant powder improved cardiac output and stroke volume, reducing total peripheral resistance in endurance-trained subjects at rest. Similarly, Cook et al., (2017) reported a dose-response effect at rest when consuming 300, 600 and 900 mg of New Zealand blackcurrant extract (NZBC) for 7 days. It is not clear if these benefits persist during exercise. Two studies reported some beneficial effects during typing exercise (Matsumoto et al., 2005) and sustained isometric contractions (Cook et al., 2017) and one study showing no effects during submaximal cycling (Willems et al., 2015). However, these studies were conducted in the morning after light breakfast (Willems et al., 2015; Cook et al., 2017), or under food restrictions avoiding polyphenol intake (Matsumoto et al., 2005; Rodriguez-Mateos et al., 2013) and examined only once the effects of anthocyanins against placebo.

However, in real-life scenarios, athletes will consume foods and sports supplements before competition and there might be occasions where they are required to race multiple times over short period (Burke, 2017). The effects of anthocyanin intake on cardiovascular parameters are unknown when being in a fed state . To address this, we examined the acute and short-term effects (4 and 7 days) of two dosages (300 and 600 mg) of NZBC extract on cardiovascular responses during rest and submaximal cycling in endurance fed trained cyclists. We hypothesized that intake of NZBC would improve cardiovascular activity at rest and during exercise mainly through an increment in cardiac output and stroke volume with a reduction in total peripheral resistance in a dose-dependent manner.

Materials and Methods

Participants

Thirteen male endurance-trained cyclists (age 39 ± 10 years, height 178 ± 7 cm, weight 75 ± 6 kg, body fat 18 ± 4%, $\dot{V}O_{2max}$ 55.3 ± 6.7 ml·kg^{-1}·min^{-1}, W_{max} 372 ± 53 Watts) volunteered. Power analysis indicated that a sample size of 13 would allow detection of a moderate effect size (d=0.4) in cardiovascular function with a high statistical power (1–β =0.80; 0.05=α level). Participants were included in the study if healthy with more than one year of cycling club experience, cycling 8-10 hrs a week, not being involved in a structured training program, and not taking nutritional supplements. Before starting, participants provided written informed consent and completed a food frequency questionnaire for calculation of total anthocyanin intake using the phenol explorer database (Neveu et al., 2010). Anthocyanin intake was 46 ± 13 mg·day^{-1}. The study was approved by the University of Chichester Research Ethics Committee (code: 1718_30, approval date: 09 February 2018) with procedures conformed to the 2013 Declaration of Helsinki.

Study design

The study design was a randomized, double-blind, control trial to examine the effects of two doses of NZBC extract (300 or 600 mg) on cardiovascular parameters. Randomization and capsule preparation were performed by METW using http://www.randomization.com. The study involved 11 visits consisting of two familiarization sessions and in three separate weeks the three visits per week for each condition. In the first visit, the relationship between power output and oxygen uptake was determined using an incremental intensity cycling test. During the second visit, maximal oxygen uptake ($\dot{V}O_{2max}$) and maximal work rate (W_{max}) were

123 determined. The three visits for each condition were performed on day one (D1), D4 and D7.
124 The recordings were taken at the same time of the day (± 2h) for each participant to minimize
125 circadian variation. At the beginning of each visit, participants were weighted then rested for
126 10 minutes in a supine position (Pickering et al., 2005). Thereafter, cardiovascular responses
127 were recorded for 20 minutes using a beat-to-beat monitoring system (Finometer® PRO,
128 Finapres Medical Systems BV, Amsterdam, The Netherlands) (details below). Participants
129 then completed an incremental cycling test , rested 15 minutes, and then cycled for 10 min
130 at sub-maximal intensity (65% $\dot{V}O_{2max}$) while cardiovascular responses were continuously
131 recorded. Finally, participants completed a 16.1 km best effort TT (Montanari et al., 2020).
132 On D7, one block was completed, and the same procedures were repeated for the remaining
133 two conditions with at least 2 weeks washout between each block (Alvarez-Suarez et al.,
134 2014). Table 1 shows the allocation, time of testing and total time to complete the study. Due
135 to the length of the study, completion time of the 11 visits was 6.6 ± 2.5 months.
136

137 ## Incremental cycling test

138 Participants cycled on a Lode ergometer (Lode BV, Groningen, Netherlands, and Ergoline, Bitz,
139 Germany). The starting power was 50 W and increased by 30 W every 4 minutes, with
140 participants keeping a pedal cadence between 70 and 90 rev·min^{-1}. Within the last minute of
141 each stage, an expired air sample was collected using Douglas bags (Cranlea & Co. Bourneville,
142 Birmingham, UK) to establish the relationship between power output and oxygen
143 consumption. Blood samples were collected with a finger prick to measure lactate
144 concentration at the end of each stage (YSI 2300 Stat Plus, Yellow Springs Instruments Co.
145 Inc., Yellow Springs, USA). On the first visit, the test ended when participants reached plasma

146 lactate value ≥ 4 mmol·L^{-1}, whereas during the experimental visits, the protocol was
147 interrupted two stages below participants' onset of blood lactate accumulation of 4 mmol·L^{-1}
148 .

149

150 ## Maximal rate of oxygen uptake

151 The test started at 50 W for 4 minutes, followed by incremental steps of 30 W every minute.
152 Expired air was collected with Douglas bags during the last 3 minutes of the protocol. Maximal
153 rate of oxygen uptake was achieved if the participants attained two of the following criteria:
154 (1) blood plasma lactate ≥ 8 mmol·L^{-1}, (2) plateau in $\dot{V}O_2$ of < 2.1 ml·kg^{-1}·min^{-1} between the
155 last two collections (3) respiratory exchange ratio (RER) ≥ 1.15 (Bassett & Howley, 2000).

156

157 ## Cardiovascular measurements at rest and during submaximal exercise

158 Cardiovascular parameters were recorded using a beat to beat blood pressure monitoring
159 system (Finometer® PRO, Finapres Medical System BV, Amsterdam the Netherlands). For
160 resting measurements, participants were in a supine position. A finger cuff was placed on the
161 middle or ring finger with the arm crossed over the chest to minimize the hydrostatic height
162 difference. Data were collected over 20 minutes and averaged over 10 consecutive seconds.
163 The lowest systolic blood pressure value over 10 consecutive seconds and associated
164 measurements were taken for analysis. During submaximal exercise, the cuff was positioned
165 on the same finger and cardiovascular measurements were averaged for the last minute of
166 the 10 minutes stage and taken for analysis. The cardiovascular parameters collected
167 included: systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure

(MAP), heart rate (HR), stroke volume (SV), cardiac output (CO) and total peripheral resistance (TPR).

New Zealand blackcurrant supplementation and diet standardization

Participants consumed two capsules every day starting from D1 with the last intake on D7. Depending on the condition, intake consisted of one NZBC extract and one PLA capsule (300 mg NZBC), two NZBC extract capsules (600 mg NZBC) or two PLA capsules (0 mg NZBC). Each NZBC extract capsule contained 105 mg of anthocyanins, consisting of 35–50% delphinidin-3-rutinoside, 5–20% delphinidin-3-glucoside, 30–45% cyanidin-3-rutinoside, and 3–10% cyanidin-3-glucoside (CurraNZ™, Health Currancy Ltd, Surrey, UK) whereas PLA contained 300 mg microcrystalline cellulose M102. Capsules were taken in the morning at breakfast except on the day of the experimental visits when intake was with a slice of buttered bread 2 h before arriving at the laboratory. Subsequently, participants were only allowed to consume water until the end of the experimental session. Blinding was successfully achieved via preparation of the conditions by a third-party researcher not involved in the data collection and analysis. Capsules were packed in pairs in single sealed plastic bags before handling to the participants who returned the empty plastic bags at the end of each block. None of the subjects could guess which condition they were taking during the study.

For each session, participants avoided strenuous exercise for 48 h, and did no exercise and had no alcohol intake 24 h before each visit. Caffeine and energy drinks were not allowed for 12 h prior the test. Lastly, prior to the first experimental visit, participants recorded their food intake for 24 h before and the same diet was replicated before each subsequent experimental visit. This method was selected to lower the participant's burden throughout the study (Jeacocke & Burke, 2010). The diet was checked for adherence and compliance was 100%.

Statistical analysis

Statistical analysis was completed using SPSS 23.0 (SPSS, Chicago, USA). The study was designed to allow a detection of 2–3 % difference in 16.1 km time-trial performance (1 − β =0.80: 0.05 =α level) (Cook et al., 2015). Data on blood lactate, substrate oxidation and TT performance are reported elsewhere (Montanari et al., 2020). Cardiovascular data at rest and during submaximal exercise were checked for homogeneity with the Mauchly test of sphericity and adjusted with the Greenhouse-Geisser test if violations were present. Normal distribution was assessed with the Shapiro-Wilk test. A RM ANOVA using a dose (PLA vs 300 vs 600 $mg \cdot day^{-1}$) by time point (D1, D4 and D7) was implemented to investigate main effects for time dose and interaction. Pairwise comparisons were analysed using the least significance post-hoc test (LSD). Data of one participant was excluded from the cardiovascular responses during sub-maximal exercise due to recording errors. If main effects or interaction were observed, data are explored reporting mean difference and 95% confidence intervals (CI). The disposition of the mean difference in relation to the small worthwhile change (SWC) were investigated. The repeated data from PLA were averaged to determine the coefficient of variation (CV) and SWC for each cardiovascular parameter. The SWC was calculated multiplying the SD by 0.6 to account for higher variability of physiological parameters (Barroso et al., 2019). All data were reported as mean ± SD unless stated otherwise. Effect size was interpreted using partial Cohen's *d* values, with small (0.2) medium (0.5) and large (0.8) effect (Cohen, 1988).

Results

NZBC extract and cardiovascular responses at rest

Table 2 shows the data for the cardiovascular measurements at rest. There was no effect for time , condition or interaction for SBP, DBP and MAP. Similarly, no differences were observed for HR, SV, CO, and TPR for time condition and interaction effect (Table 2).

NZBC extract and cardiovascular responses during sub-maximal exercise

Table 3 shows the average, CV and SWC for each cardiovascular response for PLA over the three tests compared to the observations on D1, D4 and D7 for 300 and 600mg.

Data for SBP, DBP, MAP and HR showed no main effect for time, condition or interaction (Table 3). A significant effect for time was observed for CO ($F_{(2,22)}$=5.11, p=0.015) with a medium effect size (d=0.68). Similarly, stroke volume and TPR showed a time effect ($F_{(2,22)}$=7.49, p=0.003, d=0.81; $F_{(2,22)}$=6.23, p=0.007, d=0.75, respectively) with no condition or interaction effects. Figure 1 shows the mean difference and 95% CI of 300 and 600 mg against PLA for SV, CO and TPR over the 3 days of testing. On D1, SV mean difference for 300 mg was lower than the SWC (-11.4 ml, 95%CI: -19.81, 2.97; d=0.55, p=0.013), and accompanied by a lower CO (-1.22 $L \cdot min^{-1}$, 95%CI: -2.12, 0.31; d= 0.42, p=0.013) and an increment in TPR (0.56 $mmHg \cdot min \cdot mL^{-1}$, 95%CI: -0.41, 1.55; d=0.18, p=0.48). This resulted in a decrement in SV and CO of 10 and 7%, respectively. A similar response was observed on D1 after intake of 600 mg with lower SV (-9.6 ml, 95%CI: -19.75, 0.58; d=0.43, p=0.06), CO (-1.35 $L \cdot min^{-1}$, 95%CI: -2.77, 0.06; d=0.42, p=0.06) (both -8%) with an average increment of 0.68 $mmHg \cdot min \cdot mL^{-1}$ for TPR (95%CI: -0.85, 2.21; d=0.21, p=0.46). On D4, all the cardiovascular parameters were within the range accounting for the SWC although TPR for 600 mg was close to the lower bound of

239 the SWC (-0.43 mmHg·min·mL^{-1}, 95%CI: -1.69, 0.81; d=0.16, p=0.46). On D7 consuming 600
240 mg raised SV (3.08 ml, 95%CI: -2.08, 8.26; d=0.16, p=0.21) CO (0.39 L·min^{-1}, 95%CI: -1.39, 0.60;
241 d=0.14, p=0.40) (both +2.5%) and lowered TPR by 6.5% (-0.46 mmHg·min·mL^{-1}, 95%CI: -1.80,
242 0.89; d=0.18, p=0.46), whereas intake of 300 mg did not provide any positive change (Figure
243 1).

244 Discussion

245 This is the first study to examine the intake duration and dose-response effects of NZBC
246 extract on cardiovascular responses at rest and during sub-maximal exercise in fed trained
247 cyclists. In contrast to our initial hypothesis, NZBC failed to improve cardiovascular function
248 at rest when compared to placebo. When we compared the effects of NZBC on cardiovascular
249 function during sub-maximal exercise, we observed a decrement in SV and CO on D1 with
250 both doses whereas only 600 mg raised SV and CO while lowering TPR on D7. However, the
251 interpretation of these results needed careful consideration considering the strength and
252 limitation of the current study design.

253 In order to test the intake duration, participants performed a series of repeated tests over
254 one week to examine acute and short-term (4 and 7 days) cardiovascular responses. The
255 primary finding was that neither 300 nor 600 mg improved cardiovascular function at rest
256 over this timeframe. These results are in contrast to some studies. Matsumoto et al., (2005)
257 showed that acute intake of blackcurrant anthocyanins (17 mg·kg body weight^{-1}) increased
258 blood flow by 1.22 (0.13)-fold 2h post intake. In addition, Rodriguez-Mateos et al. (2013),
259 demonstrated that acute anthocyanin intake, not only increased blood flow by 2.4 ± 0.5% at
260 1 h, and 1.5 ± 0.4% at 2 h post intake, but this response was dose-dependent reaching a

plateau once participants consumed 310 mg of blueberry anthocyanins. Although we did not measure blood flow in the present study, we did not find changes in cardiovascular responses at rest following acute consumption of NZBC extract providing 105 and 210 mg of blackcurrant anthocyanins. Considering that the plateau effect observed by Rodriguez-Mateos et al. (2013) was 310 mg of anthocyanins, it is possible that the doses used were too low to produce a significant change in cardiovascular responses. Other reasons might be related with the participants' condition (fed vs fasted), the control of the diet (polyphenol restriction vs normal diet habits) and the fitness status. It is known that endurance training improves cardiovascular responses at rest, lowering HR and blood pressure and increasing NOS expression (Green et al., 2016). Since endurance trained participants were recruited in the present study, it is possible that their cardiovascular function was already sufficiently adapted and therefore no significant changes were observed at rest. After ingestion, anthocyanins are quickly metabolised and excreted, therefore they present poor bioavailability (~12%) (Czank et al., 2013). Nevertheless, their metabolites are still present in the plasma up to 48 h (de Ferrars et al., 2014). These metabolites are biologically active. In vitro studies showed that they can increase endothelial function reducing superoxide levels and increasing endothelial heme oxygenase-1 (Edwards et al., 2015), an enzyme reported to inhibit NADPH oxidase function (Jiang et al., 2006). Therefore, a short-term intake (≥ 48 h) should allow a build-up of the metabolites in the system. However, we did not find differences between conditions and within one week of NZBC extract intake on cardiovascular responses at rest. These results are in contrast with previous research of our own group that observed improved CO by 25%, and SV by 26% with a decrease in TPR by 16% in endurance trained athletes at rest (Willems et al., 2015). Moreover, Cook et al., (2017) reported a dose-response effect for NZBC extract, with 900 mg (315 mg of anthocyanins) providing no additional benefits compared to 600 mg (210

mg of anthocyanins). Difference in outcome might be related to the time of testing. Most of our participants (10 of 13) arrived in the afternoon and they were not fasted whereas previous data were based on morning recordings after an overnight fast. Consuming a meal causes an increment in HR, SV and CO affecting the cardiovascular system up to 2 h post ingestion (Sidery and Macdonald, 1994). Cook et al., (2017) reported an average CO at rest of ~4, ~4.5 and ~4.8 $L \cdot min^{-1}$ for PLA, 300 and 600 mg of NZBC extract, respectively. In the present study, we recorded a higher average CO (>5 $L \cdot min^{-1}$) for all conditions at any time point, except for placebo intake at D7 (4.8 ± 0.9 $L \cdot min^{-1}$). Potentially, food intake close to visit time might have altered the cardiovascular response at rest. On a secondary note, we cannot exclude the intake of food interacting with anthocyanin absorption. It has been demonstrated that anthocyanin consumed with a high fat meal reached peak plasma concentration 4h post intake (Mazza et al. 2002). Using a rat model, Walton et al. (2009) reported lower plasma anthocyanin concentration when they were consumed with oats compared with water (Walton et al. 2009). We did not measure plasma anthocyanins levels, but we cannot exclude that the food consumed close to the dose on a testing day might have impaired and/or delayed anthocyanins absorption. More research in humans is warranted to better understand anthocyanins metabolism when consumed in proximity of other meals.

In the present study, some cardiovascular responses during submaximal exercise showed positive changes after seven days of intake of 600 mg of NZBC extract. Specifically, we observed small increments in SV (+3.08 ml, +2.5%) CO (+0.39 $L \cdot min^{-1}$, +2.5%) and lower TPR by 6.5% (-0.46 $mmHg \cdot min \cdot mL^{-1}$), whereas no beneficial changes were recorded for 300 mg. These data seem to support a dose relationship-effect observed in previous research (Rodriguez-Mateos et al., 2013; Cook et al., 2017). Using the same dosing strategy (600 mg), Cook et al. (2017) observed an increment of 0.6 $L \cdot min^{-1}$ and 5 ml for CO and SV respectively in

resting condition after seven days of intake with no significant difference in cardiovascular response at rest using 300 mg, therefore our results further support the intake of 600 mg of NZBC extract over the single dose.

The observations in the present study must be interpreted with caution and the following limitations. Firstly, due to the condition of our study design, most of the participants were tested in the afternoon (10 of 13) and in a fed state. It is known that the circadian rhythm has an impact on the physiological responses of our body. Cugini et al., (1993) tracked the cardiovascular activity over a 24 h period showing that CO varied considerably over the day reporting a minimum of 6 $L \cdot min^{-1}$ and a maximum of 9.49 $L \cdot min^{-1}$. Similarly, SV showed a high variation with nocturnal values of 88 ml and a maximum diurnal values of 125 ml. Moreover, mean chronograms of the bioimpedance measurements showed how CO, SV, TPR, HR and blood pressure increased over time peaking in the afternoon/early evening. Therefore, it is possible that the daily variation in the cardiovascular activity might have been partially resulted from the natural rise in HR, CO an SV values potentially prohibiting blackcurrant effects. However, each participant was tested always at the same time of the day to minimize this variation. Secondly, it is worth noting that, on D1, we observed a drop in SV by 11 and 9 ml for 300 and 600 mg compared to PLA with a moderate effect size. Such a difference is considered clinically relevant (Van Wolferen et al., 2011). This result was unexpected considering that there is no evidence of negative impact on SV and other cardiovascular parameters after acute and chronic intake of NZBC extract.

In the present study, we determined a SWC of 3.8 ml, 0.5 $L \cdot min^{-1}$ and 0.36 $mmHg \cdot min \cdot mL^{-1}$ for SV, CO and TPR, respectively. However, the CV calculated over the three PLA tests showed a CV of 5% for SV and CO and 9% for TPR which translated in a potential variation of 6 ml for SV, 0.8 $L \cdot min^{-1}$ for CO and 0.66 $mmHg \cdot min \cdot mL^{-1}$ for TPR. Similar variation was reported by

Waldron et al. (2018), using the Finapres as beat-to-beat monitoring system, with a CV of ~6% for SV during treadmill walk at 5% incline. Therefore, the CV might explain some of the variability observed on D1 as well as account for the small beneficial effects observed on D7 for 600 mg. Other studies showed mixed results on the effects of NZBC extract on cardiovascular responses during exercise, reporting enhanced blood flow, CO and SV during 2 minutes of sustained isometric contraction (Cook et al., 2017) or no effects during submaximal cycling (Willems et al., 2015). The latter study showed that a sample of 9 would be needed to detect a difference of 20% in CO. For the present study, however, cardiovascular responses were not selected as primary outcomes. Data collected were part of a wider study project which included additional physiological (lactate levels, substrate oxidation) and performance parameters (16.1 km TT) as primary outcomes (Montanari et al., 2020). Therefore, our study might have been underpowered. Finally, although participants recruited were not involved in a structured training program, we did not measure the variation in training load and intensity across time to complete the whole study (6.6 ± 2.5 months). A recent study showed that participants often fail to replicate their physical activity routines before experimental trials (Chrzanowski-Smith et al., 2020). Therefore, future protocol should include more objective assessments (monitoring duration and intensity) to minimise day-to-day variability in cardiovascular function using shorter study designs.

In conclusion, the intake of NZBC extract does not affect cardiovascular responses in endurance trained fed male cyclists. Potential reasons for the present findings might be related to the time of testing, the duration of the study, the condition of the participants (fed) and the variation in the measurement recordings. Further research is required to understand anthocyanins metabolism and effects on cardiovascular responses using ecologically valid study designs.

Novelty Statement

• This is the first study examining the effects of New Zealand blackcurrant extract on cardiovascular responses at rest and during sub-maximal exercise after acute and short-term (4 and 7 days) intake in fed trained cyclists. New Zealand blackcurrant extract did not improve cardiovascular activity under these conditions.

Practical Applications

• If a supplementation protocol is considered by athletes and practitioners, 600 mg of New Zealand blackcurrant extract might provide trivial benefits after 7 days of intake without negative effects.

• Supplementation of New Zealand blackcurrant extract should be carefully planned to avoid unknown interaction with other food matrixes that might affects its metabolism.

Acknowledgments

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Table 1: Allocation, time of testing and time to complete the study. 1 = 300 mg; 2 = 600 mg; 3 = PLA.

Participant	Allocation	Time of testing	Completion time (months)

N1	1/2/3	6pm	8.5
N2	2/1/3	6pm	8
N3	3/2/1	3pm	8.5
N4	3/1/2	6pm	8.5
N5	3/2/1	9am	7
N6	2/1/3	6pm	7
N7	3/2/1	6pm	6,5
N8	3/2/1	6pm	11
N9	2/1/3	8am	3
N10	3/2/1	6pm	5
N11	1/3/2	9am	3
N12	1/3/2	1pm	7
N13	2/1/3	4pm	3

Table 2: Cardiovascular responses at rest for each condition on day 1 (D1), day 4 (D4) and day 7 (D7). Data are expressed as mean ± SD.

Condition	**D1**	**D4**	**D7**

SBP (mmHg)			
300mg	125 ± 13	130 ± 14	121 ± 14
600mg	125 ± 11	125 ± 13	122 ± 13
Placebo	121 ± 13	121 ± 14	123 ± 14
DBP (mmHg)			
300mg	69 ± 9	73 ± 8	68 ± 8
600mg	71 ± 9	69 ± 11	68 ± 9
Placebo	66 ± 11	67 ± 9	69 ± 6
MAP (mmHg)			
300mg	87 ± 11	92 ± 11	86 ± 10
600mg	90 ± 10	87 ± 13.	87 ± 12
Placebo	85 ± 13	85 ± 12	87 ± 9
HR (bpm)			
300mg	56 ± 8	56 ± 8	55 ± 7
600mg	54 ± 5	56 ± 7	55 ± 8
Placebo	57 ± 8	55 ± 11	53 ± 5
SV (ml)			
300mg	94 ± 11	94 ± 14	92 ± 13
600mg	96 ± 12	95 ± 13	94 ± 12
Placebo	97 ± 12	98 ± 14	90 ± 12
CO ($L \cdot min^{-1}$)			
300mg	5.2 ± 1.0	5.2 ± 1.1	5.0 ± 1.0
600mg	5.1 ± 0.8	5.2 ± 1.1	5.2 ± 1.0
Placebo	5.4 ± 1.0	5.4 ± 0.6	4.8 ± 0.9
TPR ($mmHg \cdot min \cdot L^{-1}$)			
300mg	17.0 ± 3.8	18.2 ± 4.3	17.3 ± 2.9
600mg	17.9 ± 3.9	17.1 ± 4.6	17.1 ± 4.5
Placebo	16.3 ± 4.6	15.9 ± 2.8	18.4 ± 3.3

538 *Note: systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), heart rate*
539 *(HR), stroke volume (SV), cardiac output (CO) and total peripheral resistance (TPR).*

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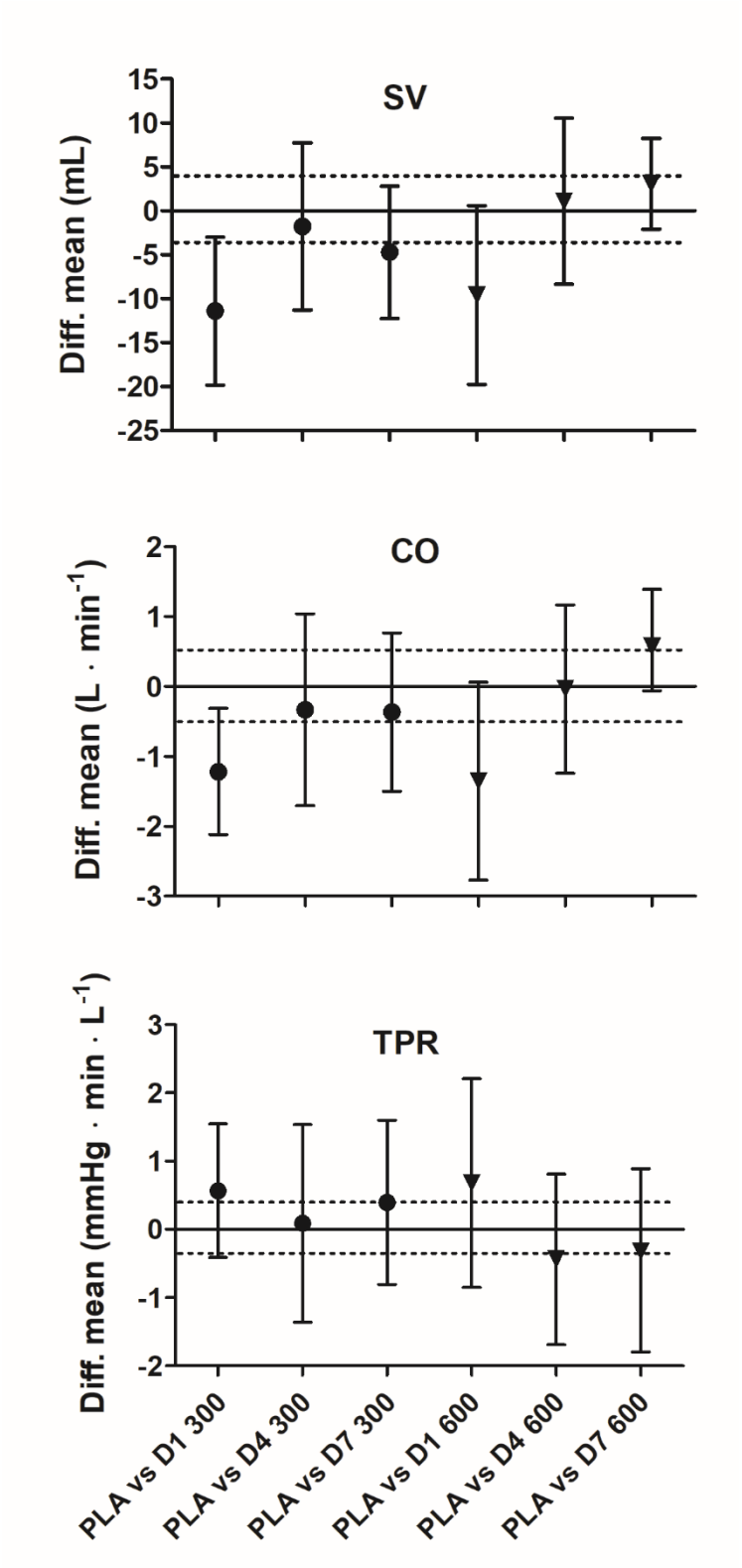

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544 **Figure 1 -** Difference in stroke volume, cardiac output and total peripheral resistance within each block for
545 placebo, 300 mg and 600 mg. Values are expresses as difference mean per cell column (95% CI).

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547

548 **Table 3:** Cardiovascular responses during sub-maximal cycling (65% $\dot{V}O_{2max}$). Placebo (PLA) data are averaged
549 over three tests on day 1 (D1), day 4 (D4) and day 7 (D7). Small worthwhile change (SWC). Coefficient of
550 variation (CV). Data are expressed as mean ± SD.

Cardiovascular responses at submaximal intensity (65% $\dot{V}O_{2max}$)				**300 mg**			**600 mg**		
Variable	**PLA**	**SWC**	**CV%**	D1	D4	D7	D1	D4	D7
SBP (mmHg)	163 ± 7	4	4	170 ± 20	165 ± 19	166 ± 20	162 ± 23	160 ± 19	165 ± 17
DBP (mmHg)	81 ± 5	3	7	88 ± 13	86 ± 10	87 ± 14	87 ± 14	83 ± 11	85 ± 11
MAP (mmHg)	108 ± 6	4	6	115 ± 17	113 ± 13	114 ± 17	113 ± 16	109 ± 13	112 ± 13
HR (bpm)	140 ± 3	2	2	143 ± 12	139 ± 12	142 ± 12	140 ± 14	140 ± 12	140 ± 12
SV (ml)	120 ± 6	3.8	5	109 ± 20	118 ± 20	115 ± 26	111 ± 23	121 ± 19	123 ± 17
CO (L·min^{-1})	16.8 ± 0.8	0.5	5	15.6 ± 2.8	16.5 ± 3.3	16.5 ± 3.8	15.5 ± 3.5	16.8 ± 2.6	17.3 ± 2.6
TPR (mmHg·min·L^{-1})	7.15 ± 0.60	0.36	9	7.72 ± 2.56	7.24 ± 2.35	7.55 ± 3.27	7.83 ± 2.86	6.72 ± 1.84	6.68 ± 1.59

551 *Note: systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), heart rate*
552 *(HR), stroke volume (SV), cardiac output (CO) and total peripheral resistance (TPR).*

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