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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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Supplementation with anthocyanin-rich blackcurrant increases blood flow, cardiac output, and stroke volume at rest. It is not known whether cardiovascular responses can be replicated over longer timeframes in fed trained cyclists. In a randomized, double-blind, crossover design, 13 male trained cyclists (age 39 ± 10 years, $\dot{V}O_2$max 55.3 ± 6.7 ml·kg$^{-1}$·min$^{-1}$) consumed two doses of New Zealand blackcurrant (NZBC) extract (300 and 600 mg/day for 1 week). Cardiovascular parameters were measured during rest and submaximal cycling (65% $\dot{V}O_2$max) on day 1 (D1), D4, and D7. Data were analyzed with an RM ANOVA using dose (placebo vs. 300 vs. 600 mg/day) by time point (D1, D4, and D7). Outcomes from placebo were averaged to determine the coefficient of variation within our experimental model, and 95% confidence interval (CI) was examined for differences between placebo 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]; $d = 0.16$, $p = .21$), cardiac output (0.39 L/min, 95% CI [−1.39, .60]; $d = 0.14$, $p = .40$) (both +2.5%), and lowered total peripheral resistance by 6.5% (−0.46 mmHg·min/ml, 95% CI [−1.80, .89]; $d = 0.18$, $p = .46$). However, these changes were trivial and fell within the coefficient of variation 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.

***Keywords:*** cardiovascular function, dose, ergometer cycling, intake duration

During endurance exercise, the increment in oxygen consumption is predominantly dictated by the metabolic demand of skeletal muscles. Cardiac output (CO) increases to meet the oxygen demand during endurance exercise (Hellsten & Nyberg, 2016). Elite athletes can sustain a high-intensity workload (85% $\dot{V}O_2$max) for several hours, with CO reaching up to 35–40 L/min (Ekblom & 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 are responsible for the red, blue, and purple colors of plant and fruits (Harborne & Grayer, 1988). A recent meta-analysis showed that acute and chronic consumption of anthocyanins can improve flow-mediated dilatation (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, an enzyme responsible for production of the vasodilator nitric oxide (Xu et al., 2004). Matsumoto et al. (2005) observed that acute intake of 17 mg/kg of blackcurrant concentrate increased forearm blood flow by 1.22 (0.13)-fold 2 hr 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 hr (de Ferrars et al., 2014). These second-phase metabolites seem to be beneficial to vascular function by increasing nitric oxide/bioavailability via reduction of nicotinamide adenine nucleotide 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 CO and stroke volume (SV), reducing total peripheral resistance (TPR) in endurance-trained subjects at rest. Similarly, Cook et al. (2017a) 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

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typing exercise (Matsumoto et al., 2005) and sustained isometric contractions (Cook et al., 2017b), and one study showed no effects during submaximal cycling (Willems et al., 2015). However, these studies were conducted in the morning after light breakfast (Cook et al., 2017a,b; Willems et al., 2015) 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 wherein they are required to race multiple times over a short period (Burke, 2017). 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 CO and SV with a reduction in TPR 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%, $\dot{V}O_2$max 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; .05 = α level). Participants were included in the study if they were healthy, had more than 1 year of cycling club experience, cycled 8–10 hr a week, were not involved in a structured training program, and were 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. The study was approved by the University of Chichester Research Ethics Committee (code: 1718_30, approval date: February 9, 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 Willems using http://www.randomization.com. The study involved 11 visits consisting of two familiarization sessions and, in three separate weeks, 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 ($\dot{V}O_2$max) and maximal work rate ($W_{max}$) were determined. The three visits for each condition were performed on day 1 (D1), day 4 (D4), and day 7 (D7). The recordings were taken at the same time of the day (±2 hr) for each participant to minimize circadian variation. At the beginning of each visit, participants were weighed, then rested for 10 min in a supine position (Pickering et al., 2005). Thereafter, cardiovascular responses were recorded for 20 min using a beat-to-beat monitoring system (Finometer® PRO, Finapres Medical Systems BV, Amsterdam, The Netherlands) (details to follow). Participants then completed an incremental cycling test, rested 15 min, and then cycled for 10 min at submaximal intensity (65% $\dot{V}O_2$max) while cardiovascular responses were continuously recorded. Finally, participants completed a 16.1-km best-effort TT (Montanari et al., 2020). On D7, one block was completed, and the same procedures were repeated for the remaining two conditions with at least a 2-week washout between each block (Alvarez-Suarez et al., 2014). Table 1 shows the allocation, time of testing, and total time to complete the study. Due to the length of the study, completion time of the 11 visits was 6.6 ± 2.5 months.

**Table 1 Allocation, Time of Testing, and Time to Complete the Study**

| Participant | Allocation | Time of testing | Completion time (months) |
|---|---|---|---|
| N1 | 1/2/3 | 6 pm | 8.5 |
| N2 | 2/1/3 | 6 pm | 8 |
| N3 | 3/2/1 | 3 pm | 8.5 |
| N4 | 3/1/2 | 6 pm | 8.5 |
| N5 | 3/2/1 | 9 am | 7 |
| N6 | 2/1/3 | 6 pm | 7 |
| N7 | 3/2/1 | 6 pm | 6,5 |
| N8 | 3/2/1 | 6 pm | 11 |
| N9 | 2/1/3 | 8 am | 3 |
| N10 | 3/2/1 | 6 pm | 5 |
| N11 | 1/3/2 | 9 am | 3 |
| N12 | 1/3/2 | 1 pm | 7 |
| N13 | 2/1/3 | 4 pm | 3 |

*Note.* 1 = 300 mg; 2 = 600 mg; 3 = PLA.

### Incremental Cycling Test

Participants cycled on a Lode ergometer (Lode BV, Groningen, Netherlands, and Ergoline, Bitz, Germany). The starting power was 50 W and increased by 30 W every 4 min, with participants keeping a pedal cadence between 70 and 90 rev/min. Within the last minute of each stage, an expired air sample was collected using Douglas bags (Cranlea & Co. Bourneville, Birmingham, United Kingdom) to establish the relationship between power output and oxygen consumption. Blood samples were collected with a finger prick to measure lactate concentration at the end of each stage (YSI 2300 Stat Plus, Yellow Springs Instruments Co. Inc., Yellow Springs). On the first visit, the test ended when participants reached plasma lactate value ≥4 mmol/L, whereas during the experimental visits, the protocol was interrupted two stages below participants' onset of blood lactate accumulation of 4 mmol/L.

### Maximal Rate of Oxygen Uptake

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

### Cardiovascular Measurements at Rest and During Submaximal Exercise

Cardiovascular parameters were recorded using a beat-to-beat blood pressure monitoring system (Finometer® PRO). For resting measurements, participants were in a supine position. A finger cuff was placed on the middle or ring finger with the arm crossed over the chest to minimize the hydrostatic height difference. Data were collected over 20 min and averaged over 10 consecutive seconds. The lowest systolic blood pressure (SBP) value over 10 consecutive seconds and associated measurements were taken for analysis. During submaximal exercise, the cuff was positioned on the same finger and cardiovascular measurements were averaged for the last minute of the 10-min stage and taken for analysis. The cardiovascular parameters collected included SBP, diastolic blood pressure, mean arterial pressure, heart rate (HR), SV, CO, and 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 placebo (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, United Kingdom), 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 hr 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 handing 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 hr and did no exercise and had no alcohol intake 24 hr before each visit. Caffeine and energy drinks were not allowed for 12 hr prior to the test. Finally, prior to the first experimental visit, participants recorded their food intake for 24 hr before, and the same diet was replicated before each subsequent experimental visit. This method was selected to lower the participants' 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 (version 23.0; SPSS, Chicago, IL). The study was designed to allow a detection of 2–3% difference in 16.1-km time-trial performance ($1 - \beta = 0.80$: $.05 = \alpha$ 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. An RM ANOVA using a dose (PLA vs. 300 vs. 600 mg/day) by time point (D1, D4, and D7) was implemented to investigate main effects for time dose and interaction. Pairwise comparisons were analyzed using the least significance post hoc test. Data of one participant were excluded from the cardiovascular responses during submaximal exercise due to recording errors. If main effects or interaction were observed, mean difference and 95% confidence interval (CI) were analysed for further comparison. The disposition of the mean difference in relation to the small worthwhile change (SWC) was 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, diastolic blood pressure, and mean arterial pressure. 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 Submaximal Exercise

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

Data for SBP, diastolic blood pressure, mean arterial pressure, and HR showed no main effect for time, condition, or interaction (Table 3). A significant effect for time was observed for CO ($F_{2,22} = 5.11$, $p = .015$) with a medium effect size ($d = 0.68$). Similarly, SV and TPR showed a time effect ($F_{2,22} = 7.49$, $p = .003$, $d = 0.81$; $F_{2,22} = 6.23$, $p = .007$, $d = 0.75$, 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]; $d = 0.55$, $p = .013$) and accompanied by a lower CO (–1.22 L/min, 95% CI [–2.12, .31]; $d = 0.42$, $p = .013$) and an increment in TPR (0.56 mmHg·min/ml, 95% CI [–.41, 1.55]; $d = 0.18$, $p = .48$). 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, .58]; $d = 0.43$, $p = .06$), CO (–1.35 L/min, 95% CI [–2.77, .06]; $d = 0.42$, $p = .06$) (both –8%) with an average increment of 0.68 mmHg·min/ml for TPR (95% CI [–.85, 2.21]; $d = 0.21$, $p = .46$). 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 the SWC (–0.43 mmHg·min/ml, 95% CI[ –1.69, .81]; $d = 0.16$, $p = .46$). On D7, consuming 600 mg raised SV (3.08 ml, 95% CI [–2.08, 8.26]; $d = 0.16$, $p = .21$) and CO (0.39 L/min, 95% CI [–1.39, .60]; $d = 0.14$, $p = .40$) (both +2.5%) and lowered TPR by 6.5% (–0.46 mmHg·min/ml, 95% CI [–1.80, .89]; $d = 0.18$, $p = .46$), whereas intake of 300 mg did not provide any positive change (Figure 1).

## Discussion

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

**Table 2 Cardiovascular Responses at Rest for Each Condition on Day 1, Day 4, and Day 7**

| Condition | Day 1 | Day 4 | Day 7 |
|---|---|---|---|
| SBP (mmHg) | | | |
| 300 mg | 125 ± 13 | 130 ± 14 | 121 ± 14 |
| 600 mg | 125 ± 11 | 125 ± 13 | 122 ± 13 |
| Placebo | 121 ± 13 | 121 ± 14 | 123 ± 14 |
| DBP (mmHg) | | | |
| 300 mg | 69 ± 9 | 73 ± 8 | 68 ± 8 |
| 600 mg | 71 ± 9 | 69 ± 11 | 68 ± 9 |
| Placebo | 66 ± 11 | 67 ± 9 | 69 ± 6 |
| MAP (mmHg) | | | |
| 300 mg | 87 ± 11 | 92 ± 11 | 86 ± 10 |
| 600 mg | 90 ± 10 | 87 ± 13. | 87 ± 12 |
| Placebo | 85 ± 13 | 85 ± 12 | 87 ± 9 |
| HR (bpm) | | | |
| 300 mg | 56 ± 8 | 56 ± 8 | 55 ± 7 |
| 600 mg | 54 ± 5 | 56 ± 7 | 55 ± 8 |
| Placebo | 57 ± 8 | 55 ± 11 | 53 ± 5 |
| SV (ml) | | | |
| 300 mg | 94 ± 11 | 94 ± 14 | 92 ± 13 |
| 600 mg | 96 ± 12 | 95 ± 13 | 94 ± 12 |
| Placebo | 97 ± 12 | 98 ± 14 | 90 ± 12 |
| CO (L/min) | | | |
| 300 mg | 5.2 ± 1.0 | 5.2 ± 1.1 | 5.0 ± 1.0 |
| 600 mg | 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·min/L) | | | |
| 300 mg | 17.0 ± 3.8 | 18.2 ± 4.3 | 17.3 ± 2.9 |
| 600 mg | 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 |

*Note.* Data are expressed as mean ± *SD*. SBP = systolic blood pressure; DBP = diastolic blood pressure; MAP = mean arterial pressure; HR = heart rate; SV = stroke volume; CO = cardiac output; and TPR = total peripheral resistance.

To test the intake duration, participants performed a series of repeated tests over 1 week to examine acute and short-term (4 and 7 days) cardiovascular responses. The primary finding was that neither 300 nor 600 mg improved cardiovascular function at rest over this timeframe. These results are in contrast to some studies. Matsumoto et al. (2005) showed that acute intake of blackcurrant anthocyanins (17 mg/kg body weight) increased blood flow by 1.22 (0.13)-fold 2 hr post intake. In addition, Rodriguez-Mateos et al. (2013) demonstrated that acute anthocyanin intake not only increased blood flow by 2.4 ± 0.5% at 1 hr and 1.5 ± 0.4% at 2 hr post intake but also that 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 to 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 nitric oxide synthase expression (Green et al., 2004). 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 metabolized and excreted, therefore they present poor bioavailability (~12%) (Czank et al., 2013). Nevertheless, their metabolites are still present in the plasma up to 48 hr (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 nicotinamide adenine dinucleotide phosphate oxidase (NADPH) function (Jiang

**Table 3 Cardiovascular Responses During Submaximal Cycling (65% $\dot{V}O_2$max)**

| Cardiovascular responses at submaximal intensity (65% $\dot{V}O_2$max) | | | | 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) | 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) | 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 |

*Note*. Data are expressed as mean ± *SD*. SBP = systolic blood pressure; DBP = diastolic blood pressure; MAP = mean arterial pressure; HR = heart rate; SV = stroke volume; CO = cardiac output; TPR = total peripheral resistance; PLA = placebo data are averaged over three tests on day 1 (D1), day 4 (D4), and day 7 (D7); SWC = small worthwhile change; CV = coefficient of variation. Data are expressed as mean ± SD.

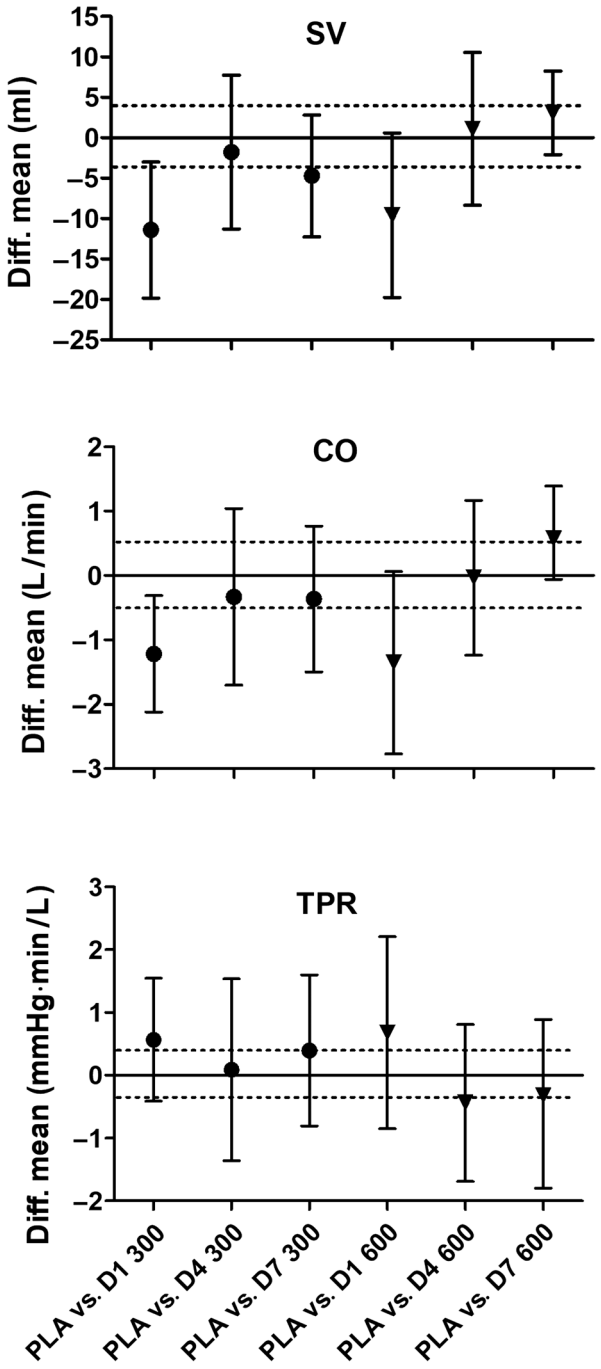


**Figure 1** — Difference in SV, CO, and TPR against PLA within each block for 300 mg (circle) and 600 mg (triangle). Dashed lines below and above 0 mark the interval for the SWC for each parameter. Values are expressed as difference mean per cell column (95% CI) (middle line). CI indicates confidence interval; CO, cardiac output; PLA, placebo; SV, stroke volume; SWC, small worthwhile change; TPR, total peripheral resistance.

et al., 2006). Therefore, a short-term intake (≥48 hr) should allow a buildup of the metabolites in the system. However, we did not find differences between conditions and within 1 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. (2017a) reported a dose–response effect for NZBC extract, with 900 mg (315 mg of anthocyanins) providing no additional benefits compared with 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 hr post ingestion (Sidery & Macdonald, 1994). Cook et al. (2017a) reported an average CO at rest of ~4, ~4.5, and ~4.8 L/min for PLA, 300 mg, and 600 mg of NZBC extract, respectively. In the present study, we recorded a higher average CO (>5 L/min) for all conditions at any time point except for placebo intake at D7 (4.8 ± 0.9 L/min). 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 4 hr post intake (Mazza et al., 2002). Using a rat model, Walton et al. (2009) reported lower plasma anthocyanin concentration when anthocyanins 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 anthocyanin absorption. More research in humans is warranted to better understand anthocyanin metabolism when consumed in proximity of other meals.

In the present study, some cardiovascular responses during submaximal exercise showed positive changes after 7 days of intake of 600 mg of NZBC extract. Specifically, we observed small increments in SV (+3.08 ml, +2.5%) and CO (+0.39 L/min, +2.5%) and lower TPR by 6.5% (–0.46 mmHg·min/ml), whereas no beneficial changes were recorded for 300 mg. These data seem to support a dose-relationship effect observed in previous research (Cook et al., 2017a; Rodriguez-Mateos et al., 2013). Using the same dosing strategy (600 mg), Cook et al. (2017a) observed an increment of 0.6 L/min and 5 ml for CO and SV, respectively, in resting condition after 7 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. First, 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 cardiovascular activity over a 24-hr period, showing that CO varied considerably over the day, reporting a minimum of 6 L/min and a maximum of 9.49 L/min. Similarly, SV showed a high variation, with nocturnal values of 88 ml and a maximum diurnal value 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 cardiovascular activity might have partially resulted from the natural rise in HR, CO, and SV values, potentially prohibiting blackcurrant effects. However, each participant was tested always at the same time of the day to minimize this variation. Second, it is worth noting that on D1, we observed a drop in SV by 11 and 9 ml for 300 and 600 mg compared with 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 an SWC of 3.8 ml, 0.5 L/min, and 0.36 mmHg·min/ml 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 into a potential variation of 6 ml for SV, 0.8 L/min for CO, and 0.66 mmHg·min/ml for TPR. Similar results were observed 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 min of sustained isometric contraction (Cook et al., 2017b) 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 that 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 the time it took 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 minimize 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 anthocyanin 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 submaximal 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 affect its metabolism.

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