Title: Intake duration of anthocyanin-rich New Zealand blackcurrant extract affects cardiovascular responses during moderate-intensity walking but not at rest

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Abstract

We examined effects of intake duration of New Zealand blackcurrant (NZBC) extract on cardiovascular responses during supine rest and moderate-intensity walking. Recreationally active men (n=15, age: 24±6 yr, BMI: 24.7±4.3 $kg \cdot m^{-2}$) volunteered in a randomized, cross-over design. One metabolic equivalent (1-MET) was measured (3.97±0.66 $mL \cdot kg^{-1} \cdot min^{-1}$) and an incremental walking test was performed to individualize speed at 4 (n=3) or 5 (n=12) METs for the 30-min walk (5.7±0.7 $km \cdot hr^{-1}$). NZBC extract (210 mg of anthocyanins) was taken with breakfast for 7 and 14 days, with a 14-days washout. The final dose was ingested 2-hr before recording of the cardiovascular responses (Portapres Model-2). At rest, %changes at 7- and 14-days intake were observed for stroke volume (+6.8% (trend), *p*=0.065; +8.5%, *p*=0.012), cardiac output (+10.1%, *p*=0.007; +8.5%, *p*=0.013), total peripheral resistance (-12.0%, *p*=0.004; -13.1%, *p*=0.011), diastolic (-5.7%, *p*=0.045; -9.7%, *p*=0.015) and mean arterial pressure (-4.4%, *p*=0.040; -7.2%, *p*=0.029), but without intake duration effect. During walking, %changes at 7- and 14-days intake were observed for stroke volume (+7.7% (trend), *p*=0.063; +9.9%, *p*=0.006), cardiac output (+8.7%, *p*=0.037; +10.1%, *p*=0.007), diastolic blood pressure (-6.2%, *p*=0.042; -10.6%, *p*=0.001), and total peripheral resistance (-9.6%, *p*=0.042; -13.5%, *p*=0.005) but without intake duration effect. During walking, %changes at 14-days were observed only for mean arterial pressure (-6.4%, *p*=0.018) and arterio-venous oxygen difference (-7.9%, *p*=0.019). NZBC extract affects cardiovascular responses at rest and during moderate-intensity exercise with 7- and 14-day intake. Only during moderate-intensity exercise, a longer intake of NZBC extract was required for an effect on some cardiovascular responses.

Keywords: Blackcurrant, blood pressure, cardiovascular responses, blood flow, anthocyanin, flavonoid, polyphenols, exercise.

Introduction

Cardiovascular diseases (CVDs) affect blood vessels and the heart and are a major cause of death worldwide. An unhealthy diet, a lack of physical activity, chronic smoking and harmful alcohol use are the main behavioural risk factors for CVDs (Barbaresko et al. 2018). With respect to the diet, an inadequate intake of fruits and vegetables provides higher risk for CVDs (Miller et al. 2017). The beneficial health effects by regular fruit and vegetable consumption are strongly associated with the anti-oxidant and anti-inflammatory effects by the polyphenol content (e.g. flavonoids) (Cassidy 2018; Miller et al. 2017). For example, a 16-yr follow-up study of 34489 postmenopausal women observed an inverse relationship between flavonoids intake (i.e. flavanones and anthocyanidins) and CVD, coronary heart disease, and all-cause mortality (Mink et al. 2007). Flavonoids are a subgroup of polyphenols and plant-based phytochemicals that cannot be synthesized by the human body (McCullough et al. 2012). Effects of intake of flavonoid-rich foods and beverages on CVDs have been linked with decreased total and LDL-cholesterol levels (Aptekmann and Cesar 2013), increased HDL-cholesterol levels (Kurowska et al. 2000), reduced platelet aggregation (Guerrero et al. 2005), reduced expression of endothelial adhesion molecules(Ludwig et al. 2004), and increased endothelium-dependent vasodilation (Ziberna et al. 2013).

Blackcurrant (Ribes nigrum L.) is a berry composed primarily of the flavonoid anthocyanin with delphinidin-3-*O*-rutinoside, delphinidin-3-*O*-glucoside, cyanidin-3-*O*-rutinoside and cyanidin-3-*O*-glucoside taking up about 95% of blackcurrant anthocyanins (Kähkönen et al. 2003). Blackcurrant anthocyanins may affect cardiovascular responses by increasing endothelium-dependent vasodilation by endothelial nitric oxide (NO) levels. For example, in an *in vitro* study by Horie et al. (2019), exposure of human vascular endothelial cells to 0.5 or 1.0 $\mu g \cdot mL^{-1}$ blackcurrant extract or 10 µM blackcurrant anthocyanins increased

the endothelial nitric oxide synthase (eNOS) expression and NO synthesis. In addition, Edirisinghe et al. (2011) observed that blackcurrant juice (1.0 μg·mL^{-1}) can increase eNOS expression by activating redox-sensitive phosphatidylinositol-3/protein kinase B pathway in vascular endothelial cells.

In humans, blackcurrant-induced cardiovascular responses were observed by Willems et al. (2015) in endurance-trained athletes. During supine rest, 7-days intake of NZBC powder (6 g·day^{-1} with 138.6 mg of anthocyanins) increased cardiac output (26%) and stroke volume (25%) with decreases in total peripheral resistance (16%). No changes were observed in heart rate and blood pressure during supine rest in addition to an absence of effects on exercise-induced cardiovascular responses at 40%, 50%, 60%, 70%, and 80% of $\dot{V}O_{2max}$ (Willems et al., 2015). Moreover, Cook et al. (2017a) examined the dose-response of 7-days intake of NZBC extract (i.e. 300 mg, 600 mg, 900 mg·day^{-1} with 105, 210 and 315 mg anthocyanins) on cardiovascular responses in endurance-trained male cyclists during supine rest. With intake of 600, and 900 mg·day^{-1} NZBC extract, an increased cardiac output by 15% and 28% and stroke volume by 7% and 18%, a decreased total peripheral resistance by 20% and 20% and mean arterial pressure by 8% and 6% was observed. In addition, Cook et al. (2017b) observed decreased total peripheral resistance, systolic, diastolic, and mean arterial blood pressure by 25%, 12%, 9% and 13%, respectively and increased cardiac output, stroke volume and femoral artery diameter by 12%, 7% and 8.2%, respectively during a submaximal (i.e. 30%) 2-min isometric voluntary contractions of the quadriceps after 7-days intake of 600 mg·day^{-1} NZBC extract. Studies with 7-days intake of New Zealand blackcurrant have provided support for the beneficial effects of intake of anthocyanin-rich foods on cardiovascular responses at rest and during exercise.

Şahin et al (2021) observed that longer intake duration of NZBC extract (i.e. 14 days) affected walking-induced metabolic responses more than 7-days intake. In a study with

blueberries, it was observed that two weeks intake was required to achieve a sustained improvement flow-mediated dilation indicating improved endothelial function (Rodriguez-Mateos et al. 2019). It is not known whether there are intake duration effects of NZBC extract on cardiovascular responses. Therefore, the present study examined the effects of intake duration of anthocyanin-rich NZBC extract (i.e. 7-days and 14-days) on cardiovascular responses during supine rest and moderate-intensity exercise. Studies on intake duration by an anthocyanin-rich supplementation may inform optimal dosing strategies to obtain health benefits.

Materials and methods

Participants

Fifteen recreationally active healthy men (age: 24±6 years, body mass: 79±16 kg, height: 178±6 cm; body fat: 15.1±5.1%, BMI: 24.7±4.3 $kg \cdot m^{-2}$) volunteered and provided written informed consent. Participants were non-smokers, not having known allergy to berries or berry products and advised not to take other supplementation during the study. A randomised, cross-over experimental design was used for the study and ethical approval was obtained from the University of Chichester Research Ethics Committee (ethical approval code: 1718_34) with protocols and procedures conformed to the 2013 Declaration of Helsinki. This trial was registered at www.clinicaltrials.gov as NCT05067062.

Experimental design

Participants had four morning laboratory visits. Participants were instructed to refrain from caffeine and alcohol intake for 24 hours and abstain from vigorous exercise for 48 hours before each laboratory visit. In the first visit, height, body mass, and body fat% (Tanita BC418 Segmental Body Composition analyzer, Tanita, IL, USA) were measured. A food

frequency questionnaire with anthocyanin-containing foods and drinks listed in the Phenol Explorer database (Neveu et al. 2010) was completed by participants to estimate daily anthocyanin intake (86±74 mg·day^{-1}). The physical activity level of participants was quantified (4534±1576 MET·week^{-1}) with the short version of the International Physical Activity Questionnaire (Lee et al. 2011). Figure 1 provides a schematic diagram of the experimental design and measurements during the four visits.

Participants were required to lie supine on a massage table for the resting measurements of cardiovascular responses and oxygen consumption after being seated in a chair for 10 min. Expired air was collected for two times for 10 min with Douglas bags. Expired air was analysed with a three-point calibrated Servomex gas analyser (Series 1400, Crowborough, UK) and volume measured (Harvard Apparatus Ltd. Dry gas meter). Gas volumes were calculated using Haldane transformation and standardization to STPD conditions with consideration of inspired fractions of oxygen and carbon dioxide within the laboratory during expired air collection. For the two expired air collections during supine rest, the lowest oxygen uptake was taken to represent the one-metabolic equivalent (1-MET, $\dot{V}O_2$: 3.97±0.66 mL·kg^{-1}·min^{-1}). After the resting measurements, the participants performed an incremental walking protocol on a treadmill (HP Cosmos Pulsar Bodycare Products UK) at speeds of 2, 3, 4, 5, and 6 km·hr^{-1}, each speed for 8 minutes with a 2-min break between each speed. Expired air was collected in the last 3 min of each speed. The incremental treadmill walking protocol was performed to individualize the participant's walking speeds at the moderate-intensity of 4 or 5 METs according to the linear relationship between walking speed and oxygen uptake. For three participants, the moderate-intensity exercise for examination of cardiovascular responses (see below) was taken at 4 MET as 5 MET speed would require jogging. Visits 2, 3 and 4 (were for recording of cardiovascular responses during supine rest and moderate-intensity exercise.

Visits for intake duration effects

Participants visited the laboratory for 3 different conditions: 1) control (no supplementation), 2) 7-days NZBC extract intake and 3) 14-days NZBC extract intake. For the 7- and 14-days intake conditions, two capsules of NZBC extract (600 mg containing 210 mg of anthocyanins, i.e. 35–50% delphinidin-3-*O*-rutinoside, 5–20% delphinidin-3-*O*-glucoside, 30–45% cyanidin-3-*O*-rutinoside, 3–10% cyanidin-3-*O*-glucoside) (CurraNZ, Health Currancy Ltd., Surrey, UK) were consumed every morning with breakfast. Participants consumed the last 2 capsules two hours before the visits and had one slice of bread and water 3 hours before the visits. A 14-day washout period was applied between each visit to eliminate previous supplementation effect (Alvarez-Suarez et al. 2014). No adverse reactions by intake of New Zealand blackcurrant extract were reported by the participants. The participants recorded 48 hours food diary before visit 2 and replicated it for 48 hours prior to visit 3 and 4. Food diaries were analysed using Nutritics (Nutritics LTD., Dublin, Ireland) in carbohydrate, fat, protein, and total energy intake.

The experimental protocol consisted of measurement of cardiovascular responses during supine rest for 2x10 min and during 30 min of moderate-intensity walking. Cardiovascular measurements were obtained with a beat-to-beat blood pressure monitoring system (Portapres® Model 2, Finapres Medical Systems BV, Amsterdam, The Netherlands) using the arterial volume clamp method (Penaz 1973). The Portapres® is a beat-to-beat finger pressure analyser that allows the non-invasive continuous measurement of hemodynamic parameters (Eckert and Horstkotte 2002). The finger cuff was positioned around the same finger of the left hand for each testing and heart rate, stroke volume, cardiac output, systolic blood pressure, diastolic blood pressure, mean arterial pressure and total peripheral resistance were derived. In addition, the rate pressure product (i.e. heart rate x systolic blood pressure) and the

arteriovenous oxygen difference (i.e. oxygen uptake divided by cardiac output) were calculated (Bhambhani et al. 1997).

During supine rest, cardiovascular responses were obtained two times for 10 min with Portapres® and expired air was collected with Douglas bags to determine oxygen uptake. Cardiovascular responses during the 10 min with the lowest minute ventilation were taken as resting cardiovascular responses. For the 30-min moderate-intensity exercise, participants walked on the treadmill at a speed of 4 (n=3) or 5 (n=12) METs (average speed: 5.7±0.7 $km \cdot hr^{-1}$) and cardiovascular responses and oxygen uptake were measured at 7–10, 17–20 and 27–30 min during the walk and averaged.

Statistical analysis

Statistical analyses were completed using Graphpad Prism (GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California, USA). Absolute values of the cardiovascular responses for the three conditions (i.e. control, 7-days and 14-days) during supine rest and during moderate-intensity exercise were analysed using one-way repeated measures ANOVA with post-hoc Tukey test. When the ANOVA provided a $p<0.05$, the absolute changes and %changes of the responses for the 7-days and 14-days conditions were tested against zero with a one-sample t-test. In addition, the absolute changes and %changes of the responses for the 7-days and 14-days conditions were analysed with a paired student t-test. Effect sizes (Cohen's d) are reported for the absolute values at 7-days and 14-days when the ANOVA provided a $p<0.05$. Cohen's d effect sizes were calculated and considered trivial ($d<0.2$), small ($d=0.2$-0.49), moderate ($d=0.5$-0.79) and large ($d \geq 0.8$), respectively (Cohen et al 1988). The sample size of 15 participants was higher or similar to previous studies with observations of effects of NZBC extract on cardiovascular responses [e.g. Willems et al 2015 (n=13); Cook et al. 2017a (n=15)]. Significance was accepted at $p<0.05$ with $0.05 \geq p \leq 0.1$

interpreted according to guidelines by Curran-Everett and Benos (2004). All data are reported as mean±SD.

Results

There were no differences for carbohydrate, fat, protein and total energy intake between the control and 7-days and 14-days intake conditions with New Zealand blackcurrant extract (Table 1).

Cardiovascular responses during supine rest

Heart rate

During supine rest, intake of anthocyanin-rich New Zealand blackcurrant extract for 7-days and 14-days had no effect on heart rate (one-way ANOVA: p=0.276; control: 61±10, 95% CI [56, 66 beats·min^{-1}]; 7-days: 63±11, 95% CI [56, 69 beats·min^{-1}]; 14-days: 61±10, 95% CI [56, 66 beats·min^{-1}]; Figure 2A).

Stroke volume

Higher stroke volume was observed only with 14-days intake of New Zealand blackcurrant extract (Figure 2B) (one-way ANOVA, p=0.048; control: 95±13, 95% CI [88, 102 mL]; 7-days: 101±15, 95% CI [92, 109 mL], p>0.05, d=0.43; 14-days: 103±18, 95% CI [93, 113 mL], p<0.05, d=0.51). However, the absolute changes for stroke volume against zero showed a trend for higher values at 7-days (p=0.082) and for 14-days a change (p=0.011) but these were not different from each other (p=0.57). In addition, %changes for stroke volume against zero were higher by 6.8% (trend, p=0.065) and 8.5% (p=0.012) for 7-days and 14-days intake and not different from each other (p=0.65), indicating no intake duration effect.

Cardiac output

Only cardiac output by 7-days intake of New Zealand blackcurrant extract was higher by 10.1% (Figure 2C) (one-way ANOVA, p=0.025; control: 5.68±0.71, 95% CI [5.29, 6.07 $L \cdot min^{-1}$]; 7-days: 6.23±0.89, 95% CI [5.74, 6.72 $L \cdot min^{-1}$], p<0.05, d=0.68; 14-days: 6.14±0.88, 95% CI [5.65, 6.63 $L \cdot min^{-1}$], p>0.05, d=0.58). However, the absolute changes of cardiac output against zero were different at 7-days (p=0.006) and 14-days (p=0.018) but these were not different from each other (p=0.71). In addition, %changes for cardiac output against zero were higher by 10.1% (7-days, p=0.007) and 8.5% (14-days, p=0.013) and not different from each other, indicating no intake duration effect.

Systolic blood pressure

During supine rest, intake of New Zealand blackcurrant extract had no effect on systolic blood pressure (one-way ANOVA: p=0.115; control: 137±13, 95% CI [130, 145 mmHg]; 7-days: 134±16, 95% CI [125, 143 mmHg]; 14-days: 130±15, 95% [122, 139 mmHg]) (Figure 2D).

Diastolic blood pressure

Diastolic blood pressure was lower by 9.7% with 14-days intake (Figure 2E) (one-way ANOVA: p=0.010; control: 70±7, 95% CI [66, 74 mmHg]; 7-days: 66±8, 95% CI [62, 70 mmHg], p>0.05, d=-0.53; 14-days: 63±9, 95% CI [58, 68 mmHg], p<0.05, d=-0.87). However, the absolute changes for diastolic blood pressure against zero were different at 7-days (p=0.032) and 14-days (p=0.014) but these were not different from each other (p=0.181). In addition, the %changes for diastolic blood pressure against zero were lower by 5.6% (p=0.045) and 9.7% (p=0.015) and not different from each other, indicating no intake duration effect.

Mean arterial pressure

Mean arterial pressure was lower by 7.2% with 14-days intake (Figure 2F) (one-way ANOVA: p=0.019; control: 87±7, 95% CI [83, 91 mmHg]; 7-days: 83±8, 95% CI [79, 88

mmHg], p>0.05, d=-0.53; 14-days: 81±9, 95% CI [76, 86 mmHg], p<0.05, d=-0.74). However, the absolute changes for mean arterial pressure against zero were different at 7-days (p=0.033) and 14-days (p=0.029) but these were not different from each other (p=0.22). In addition, the %changes of mean arterial blood pressure against zero were lower for 7-days by 4.4% (p=0.040) and for 14-days by 7.2% (p=0.029), suggesting no intake duration effect.

Total peripheral resistance

Total peripheral resistance was lower with 7-days intake by 12.0% and by 13.1% with 14-days intake (Figure 2G) with no intake duration effect (one-way ANOVA, p=0.005; control: 15.7±2.9, 95% CI [14.1, 17.3 mmHg·min·L^{-1}]; 7-days: 13.6±2.5, 95% CI [12.3, 15.0 mmHg·min·L^{-1}], p<0.05, d=-0.76; 14-days: 13.4±2.6, 95% [12.0, 14.9 mmHg·min·L^{-1}], p<0.05, d=-0.82). The absolute changes for total peripheral resistance against zero were different at 7-days (p=0.004) and 14-days (p=0.008), but not different from each other (p=0.82). In addition, the %changes for total peripheral resistance against zero were lower by 12% at 7-days (p=0.004) and by 13.1% at 14-days (p=0.011) and not different from each other (p=0.082), suggesting no intake duration effect.

Rate-pressure product

During supine rest, intake of New Zealand blackcurrant extract had no effect on the rate pressure product (Figure 2H) (one-way ANOVA: p=0.209; control: 8416±1278, 95% CI [7708, 9124 mmHg·bpm]; 7-days: 8216±1783, 95% CI [7228, 9203 mmHg·bpm]; 14-days: 7837±1463, 95% CI [7027, 8647 mmHg·bpm].

Arterio-venous oxygen difference

Arterio-venous oxygen difference showed a trend (one-way ANOVA: p=0.087) but without significance for the Tukey post-hoc testing (Figure 2I) (control: 5.24±1.05, 95% CI [4.66, 5.82 mL·100 mL^{-1}], p>0.05, d=-0.53; 7-days: 4.72±0.88, 95% CI [4.23, 5.21 mL·100 mL^{-1}]; 14-days: 4.80±1.25, 95% CI [4.11, 5.49 mL·100 mL^{-1}], p>0.05, d=-0.38). However, only the

absolute difference for arterio-venous oxygen difference against zero was different at 7-days (p=0.038) and not at 14-days (p=0.118). In addition, the %changes for arterio-venous oxygen difference against zero was lower with a trend at 7-days (p=0.068) but not different for 14-days (p=0.150).

Cardiovascular responses during moderate-intensity walking

Heart rate

During moderate-intensity walking, intake of anthocyanin-rich New Zealand blackcurrant extract had no effect on heart rate (one-way ANOVA: p=0.820; control: 103±19, 95% CI [92, 113 beats·min^{-1}]; 7-days: 104±19, 95% CI [93, 114 beats·min^{-1}]; 14-days: 103±18, 95% CI [93, 113 beats·min^{-1}]; Figure 3A).

Stroke volume

Higher stroke volume was observed with 14-days intake (Figure 3B) (one-way ANOVA, p=0.017; control: 114±13, 95% CI [107, 121 mL]; 7-days: 123±22, 95% CI [111, 135 mL], p>0.05, d=0.50; 14-days: 126±21, 95% CI [114, 137 mL], p<0.05, d=0.69). However, the absolute changes for stroke volume against zero were different at 7-days (p=0.049) and 14-days (p=0.009) but these were not different from each other (p=0.53). In addition, the %changes for stroke volume against zero were lower with a trend by 7.7% (p=0.063) and lower by 9.9% (p=0.006), but these were not different from each other (p=0.51) suggesting no intake duration effect.

Cardiac output

Cardiac output by 7- and 14-days intake of anthocyanin-rich New Zealand blackcurrant intake was higher (Figure 3C) with no intake duration effect (one-way ANOVA, p=0.012; control: 11.65±1.96, 95% CI [10.56, 12.73 L·min^{-1}]; 7-days: 12.67±2.52, 95% CI [11.28, 14.07 L·min^{-1}], p<0.05, d=0.45; 14-days: 12.74±2.11, 95% CI [11.57, 13.90 L·min^{-1}], p<0.05, d=0.54). In

addition, the absolute changes against zero for cardiac output were different at 7-days (p=0.020) and 14-days (p=0.011) but these were not different from each other (p=0.87). In addition, the %changes for cardiac output against zero were higher by 8.7% at 7-days (p=0.037) and by 10.1% at 14-days (p=0.007) but not different from each other (p=0.71), suggesting no intake duration effect.

Systolic blood pressure

During moderate-intensity walking, intake of New Zealand blackcurrant extract had no effect on systolic blood pressure (one-way ANOVA: p=0.319; control: 158±18, 95% CI [148, 168 mmHg]; 7-days: 153±14, 95% CI [145, 161 mmHg]; 14-days: 153±16, 95% [144, 162 mmHg], Figure 3D).

Diastolic blood pressure

Diastolic blood pressure was lower with 14-days intake (Figure 3E) (one-way ANOVA: p=0.002; control: 71±9, 95% CI [66, 76 mmHg]; 7-days: 66±9, 95% CI [61, 71 mmHg], p>0.05, d=-0.50; 14-days: 63±11, 95% CI [57, 70 mmHg], p<0.05, d=-0.73). However, the absolute changes for diastolic blood pressure against zero were different at 7-days (p=0.037) and 14-days (p=0.002) but these were not different from each other (p=0.15). In addition, the %changes for diastolic blood pressure against zero were lower by 6.2% at 7-days (p=0.042) and by 10.6% at 14-days (p=0.001) but not different from each other (p=0.12), suggesting no intake duration effect.

Mean arterial pressure

Mean arterial pressure was lower with 14-days intake (Figure 3F) (one-way ANOVA: p=0.034; control: 93±10, 95% CI [87, 99 mmHg]; 7-days: 89±9, 95% CI [84, 94 mmHg], p>0.05 14-days: 87±11, 95% CI [81, 93 mmHg], p<0.05, d=-0.56). The absolute differences for mean arterial pressure against zero were not changed at 7-days (p=0.122) but different at 14-days (p=0.018). In addition, the %changes for mean arterial pressure against zero were not

different at 7-days (p=0.168) but lower by 6.4% at 14-days by (p=0.018), suggesting an intake duration effect.

Total peripheral resistance

Total peripheral resistance was lower with 14-days intake (Figure 3G) (one-way ANOVA, p=0.026; control: 8.21±1.79, 95% CI [7.22, 9.21 mmHg·min·L^{-1}]; 7-days: 7.50±2.76, 95% CI [5.97, 9.03 mmHg·min·L^{-1}], p>0.05; 14-days: 7.08±1.92, 95% [6.01, 8.14 mmHg·min·L^{-1}], p<0.05, d=-0.61). The absolute changes for total peripheral resistance against zero were not changed at 7-days (p=0.12) but were changed at 14-days (p=0.004). However, the %changes for total peripheral resistance against zero was lower at 7-days by 9.6% (p=0.042), and lower by 13.5% at 14-days (p=0.005), but not different from each (p=0.431), suggesting no intake duration effect.

Rate-pressure product

During moderate-intensity walking, intake of New Zealand blackcurrant extract had no effect on the rate pressure product (Figure 3H) (one-way ANOVA: p=0.537; control: 16388±4343, 95% CI [13983, 18793 mmHg·bpm]; 7-days: 16018±3764, 95% CI [13934, 18103 mmHg·bpm]; 14-days: 15897±3970, 95% CI [13699, 18096 mmHg·bpm].

Arterio-venous oxygen difference

Arterio-venous oxygen difference showed a trend (one-way ANOVA: p=0.083) but without significance for the Tukey post-hoc testing (Figure 3I) (control: 13.21±2.48, 95% CI [11.84, 14.58 mL·100 mL^{-1}], p>0.05; 7-days: 12.40±2.65, 95% CI [10.93, 13.87 mL·100 mL^{-1}]; 14-days: 12.17±2.80, 95% CI [10.62, 13.72 mL·100 mL^{-1}], p>0.05, d=-0.39). However, the absolute change for arterio-venous oxygen difference against zero was not different at 7-days (p=0.113) but only at 14-days (p=0.029). In addition, the %changes for arterio-venous oxygen difference against zero was not different at 7-days (p=0.125) but lower by 7.3% (p=0.019), suggesting in intake duration effect.

Discussion

The present study provided novel observations on the role of intake duration of an anthocyanin-rich berry extract on cardiovascular responses at rest and during moderate intensity exercise in healthy males. During supine rest, 7-days and 14-days intake of NZBC extract enhanced cardiovascular responses but without an intake duration effect. This was in contrast with the observations by 7-days and 14-days intake of NZBC extract during moderate intensity exercise for which an intake duration effect was present for some cardiovascular responses. Future work is needed to examine whether the observations in the present study are uniquely associated with the blackcurrant anthocyanin composition, primarily cyanidins and delphinidins or whether such responses can be observed by berry supplementation with different anthocyanin composition than blackcurrant in addition to reducing the risk for cardiovascular diseases. For example, a recent systematic review and meta-analysis by Miraghajani et al (2020) reported for blueberry that the current evidence did not support modification of risk factors for cardiovascular diseases but the review did not include cardiovascular responses as observed in the present study.

Previous studies with 7-days intake of NZBC powder (Willems et al. 2015) and NZBC extract (Cook et al. 2017a) also observed increases in cardiac output and stroke volume with a decrease in total peripheral resistance and without changes in heart rate at rest. These changes on cardiovascular function by 7-days intake of anthocyanin-rich blackcurrant may occur via increased nitric oxide production in human endothelial cells (Horie et al. 2019). Blackcurrant anthocyanins can enhance nitric oxide production in endothelial cells by increasing the expression of endothelial nitric oxide synthase which is leading to endothelium-dependent vasorelaxation and vasodilation (Edirisinghe et al. 2011; Horie et al. 2019). The present study also observed a 4 mmHg decrease in diastolic blood pressure and mean arterial pressure at

rest with 7-days intake of NZBC extract. Cook et al. (2020) observed a 12 mmHg decrease in diastolic blood pressure in older adults in rest with 7-days intake of NZBC extract but no effect on blood pressure was present in trained cyclists and triathletes (Cook et al. 2017a, Willems et al. 2015). In addition, Okamoto et al (2020) observed in older Japanese adults a 10 mmHg decrease in central blood pressure with 7-days intake of NZBC extract. Future studies may address the responsiveness of participants of different age, ethnicity, training status who may benefit from a reduction in systolic blood pressure. In addition, studies with intake duration of blackcurrant longer than 2 weeks may be required to obtain reductions in systolic blood pressure at rest in participants with different backgrounds.

The moderate intensity exercise modality (i.e. between 3-6 METs) that was employed in the present study is recommended by the World Health Organization and American College of Sports Medicine as an intensity of exercise to prevent chronic diseases and obtain health benefits (Jakicic et al. 2001; WHO 2010). In the present study, increased cardiac output and stroke volume with decreased diastolic blood pressure during moderate intensity walking were observed with 7-days intake of NZBC extract. Longer intake (i.e. 2 weeks of daily 600 mg of blackcurrant anthocyanins) was required to decrease total peripheral resistance during moderate intensity walking. Therefore, our observations on the changes in cardiovascular responses during moderate intensity walking seems to suggest that potential mechanisms take weeks to allow adaptation in the cardiovascular system by intake of anthocyanin-rich blackcurrant extract. Czank et al. (2013) identified numerous anthocyanin metabolites in the plasma after ingestion of 500 mg of cyanidin-3-glucoside. The anthocyanin-derived metabolites reached maximum serum concentration between 1,8 and 15,7 hours and remained in the plasma between 12,4 and 51,6 hours after ingestion. Therefore, intake duration effects may happen due to a possible accumulation of anthocyanin-derived metabolites with more potential to alter mechanisms that affect cardiovascular responses during exercise. In addition,

the accumulation of anthocyanins in tissue themselves has been shown in animal tissues (Kalt et al. 2008; Sakakibara et al. 2009) but as far as we known this has not been examined in humans. Potential accumulation of anthocyanins in human cells may be a time-dependent process affecting a changes of cell function over time. Future work should address the plasma bioavailability of blackcurrant anthocyanin-derived metabolites and tissue presence of anthocyanins with chronic dosing. However, our observations indicate that over time, there is the development of an anthocyanin or anthocyanin-derived metabolite(s) effect that potentially lowers the presence of reactive oxygen species that are produced during the exercise. This potential antioxidant effect by intake of blackcurrant lowers the interaction of the reactive oxygen species with nitric oxide creating an environment with reduced conversion of nitric oxide. Higher availability of nitric oxide contributes then to the observed cardiovascular responses during moderate intensity walking.

Timing, dose, and intake duration are crucial to determine optimal intake of dietary supplements (Naderi et al. 2016) but optimal intake strategies for anthocyanin-rich foods or anthocyanin-rich supplementation to enhance cardiovascular responses are not yet known. Siasos et al. (2013) examined the effects of 7-days and 14-days intake of concord grape juice (7 $ml \cdot kg^{-1} \cdot day^{-1}$ with 197 mg polyphenols·100 ml^{-1} and 52 mg anthocyanin·100 ml^{-1}) on flow-mediated dilation to evaluate endothelial function and pulse wave velocity to evaluate arterial stiffness in healthy smokers (n=26, 10 male). Improvements in flow-mediated dilation and pulse wave velocity were observed before and 1-min and 20-min after smoking with 7-days and 14-days intake although the study did not seem to address whether there was an intake duration effect. However, the effects by concord grape juice are likely not completely due to the anthocyanin content as the amount of hydroxycinnamates, flavonols and flavan-3-ols were much higher than the anthocyanin content (Siasos et al. 2013). In addition, daily intake for 4 weeks of 11 g of free-dried wild blueberry powder (362 mg of polyphenols of which 150 mg

anthocyanins) achieved a sustained improvement flow-mediated dilation after two weeks indicating improved endothelial function (Rodriguez-Mateos et al. 2019). However, the present study is the first study that examines the effects of intake duration of at least two weeks of anthocyanin-rich berry extract on cardiovascular responses. Only one study (Hurst et al. 2020) dosed for 5 weeks with daily intake of 3.2 $mg \cdot kg^{-1}$ of blackcurrant anthocyanins and reported enhanced exercise recovery compared with an acute intake. However, it is not known whether such effects could have been obtained with shorter intake duration.

The present study also provided indication by lower arterio-venous oxygen difference that intake of NZBC extract can enhance tissue oxygen uptake at rest and during dynamic moderate intensity exercise. Fryer et al (in press, 2020) also provided observations of enhanced muscle oxygenation with near-infrared spectroscopy during isometric contractions of forearm skeletal muscles in climbers. It is possible that the presence of lower arterio-venous oxygen difference contributed to performance-enhancing effects in some exercise modalities by intake of NZBC extract (e.g. Cook et al. 2015). However, the potential benefits of lower arterio-venous oxygen difference, as observed in the present study is unclear. The lower arteriovenous oxygen difference may have been due to enhanced blood flow, compensating for enhanced cardiac output and not indicative of increased oxygen use due to our approach to calculate arteriovenous oxygen difference in the present study. However, the significant increase in cardiac output with 14-days intake of New Zealand blackcurrant without a lower value for arteriovenous oxygen difference may indicate an increase in oxygen uptake.

There are some limitations of the present study. First, we did not measure the plasma availability of anthocyanins and anthocyanin-derived metabolites, NO and eNOS expression levels after 7-days and 14-days NZBC intake which can provide potential causal evidence for efficiency of NZBC extract intake. Further studies should consider to evaluate intake duration

of anthocyanin-rich foods. Second, participants did not have a standard diet for the supplementation period and they recorded 48 hours food diary before the first supplementation test and replicated for the following supplementation tests. However, flavonoids can interact with macronutrients during absorption. For instance, Serra et al. (2010) showed that carbohydrate-rich food can repress the absorption of procyanidin dimers and trimers in rats. Therefore, there was a possibility to have different cardiovascular responses between participants according to variations in their normal diet. Third, it is possible that for some physiological parameters, a higher daily dose may be required or increased number of participants to reach statistical significance for the absolute values. For example, 9 out of 15 and 13 out of 15 participants had lower arteriovenous oxygen difference during the moderate-intensity walk at 7- and 14-days, but the *p*-value of the one-way ANOVA was 0.083.

It is concluded that NZBC extract enhanced cardiovascular responses at rest and during moderate intensity exercise with 7-days and 14-days NZBC intake. Only during moderate intensity exercise, a longer intake (i.e. 14-days) of NZBC extract was required for an effect on some cardiovascular responses.

Acknowledgments

We thank Health Currancy Ltd (UK) and CurraNZ Ltd (NZ) for supplements and the Scientific and Technological Research Council of Turkey for funding MA Şahin to conduct the study at the University of Chichester (UK).

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Table 1. Dietary and energy intake 48 h before each experimental visit. 7-days and 14-days daily refers to the intake conditions of New Zealand blackcurrant extract. Data was analysed with one-way repeated measures ANOVA (*p*-values are from the ANOVA).

	Control	**7-days daily**	**14-days daily**	***p*-value**
Carbohydrate (g)	428 ± 111	426 ± 120	420 ± 111	0.604
(g · kg body mass^{-1})	5.7 ± 1.9	5.6 ± 1.9	5.5 ± 1.8	0.579
Fat (g)	171 ± 67	173 ± 67	170 ± 68	0.815
(g · kg body mass^{-1})	2.2 ± 0.7	2.2 ± 0.7	2.2 ± 0.7	0.838
Protein (g)	253 ± 88	252 ± 80	244 ± 87	0.222

(g · kg body mass^{-1})		3.3 ± 1.2	3.3 ± 1.0	3.2 ± 1.2	0.240
Total energy intake (kcal)		4261 ± 937	4271 ± 992	4148 ± 981	0.269
(kcal · kg body mass^{-1})		55 ± 13	55 ± 13	54 ± 13	0.229
Total energy intake (kJ)		17826 ± 3922	17871±4149	17354 ± 4104	0.269
(kJ · kg body mass^{-1})		231 ± 55	231 ± 56	224 ± 54	0.229

Figure legends

Figure 1. Experimental design and measurements during the four visits. NZBC extract, New Zealand blackcurrant extract. 1-MET, one-metabolic equivalent. FFQ, food-frequency questionnaire. IPAQ, international physical activity questionnaire.

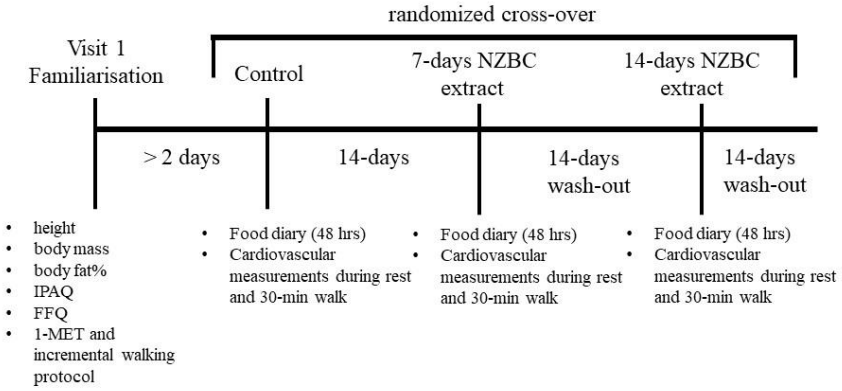

Figure 2. Heart rate (A), stroke volume (B), cardiac output (C), systolic blood pressure (D), diastolic blood pressure (E), mean arterial blood pressure (F), total peripheral resistance (G), rate-pressure product (H), and arterio-venous oxygen difference (I) during supine rest. Data reported as mean ± SD from 15 participants. 7-days and 14-days refers to the intake duration of New Zealand blackcurrant extract. Data was analysed with one-way repeated measures ANOVA with post-hoc Tukey test, * indicates difference with control (p<0.05).

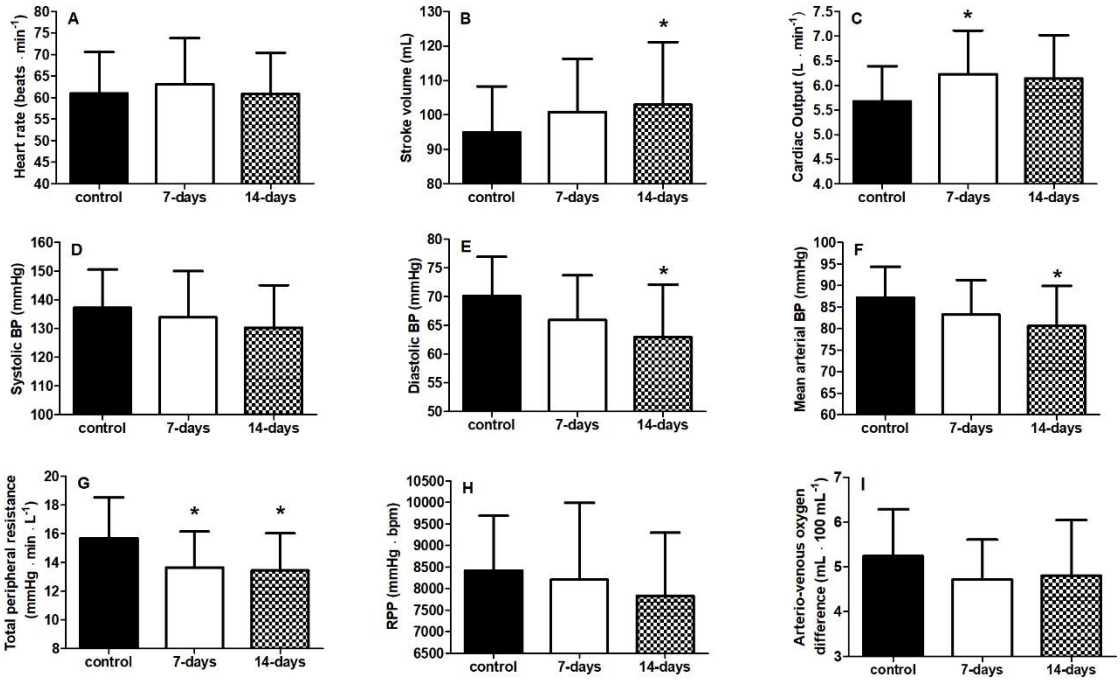

Figure 3. Heart rate (A), stroke volume (B), cardiac output (C), systolic blood pressure (D), diastolic blood pressure (E), mean arterial blood pressure (F), total peripheral resistance (G), rate-pressure product (H), and arterio-venous oxygen difference (I) during 30-min of moderate intensity walking. Data reported as mean ± SD from 15 participants. 7-days and 14-

days refers to the intake duration of New Zealand blackcurrant extract. Data was analysed with one-way repeated measures ANOVA with post-hoc Tukey test, * indicates difference with control (p<0.05).