

Northumbria Research Link

Citation: Costello, Rianne, Keane, Karen, Lee, Ben J., Willems, Mark E. T., Myers, Stephen D., Myers, Fiona, Lewis, Nathan A. and Blacker, Sam D. (2022) Plasma uptake of selected phenolic acids following New Zealand blackcurrant extract supplementation in humans. *Journal of Dietary Supplements*, 19 (5). pp. 672-688. ISSN 1939-0211

Published by: Taylor & Francis

URL: <https://doi.org/10.1080/19390211.2021.1914802>
<<https://doi.org/10.1080/19390211.2021.1914802>>

This version was downloaded from Northumbria Research Link:
<https://nrl.northumbria.ac.uk/id/eprint/46295/>

Northumbria University has developed Northumbria Research Link (NRL) to enable users to access the University's research output. Copyright © and moral rights for items on NRL are retained by the individual author(s) and/or other copyright owners. Single copies of full items can be reproduced, displayed or performed, and given to third parties in any format or medium for personal research or study, educational, or not-for-profit purposes without prior permission or charge, provided the authors, title and full bibliographic details are given, as well as a hyperlink and/or URL to the original metadata page. The content must not be changed in any way. Full items must not be sold commercially in any format or medium without formal permission of the copyright holder. The full policy is available online: <http://nrl.northumbria.ac.uk/policies.html>

This document may differ from the final, published version of the research and has been made available online in accordance with publisher policies. To read and/or cite from the published version of the research, please visit the publisher's website (a subscription may be required.)

Title: Plasma uptake of selected phenolic acids following New Zealand blackcurrant extract supplementation in humans

Authors: Dr Rianne Costello^{1,5}, Dr Karen M Keane^{2,7}, Dr Ben J Lee^{1,6}, Professor Mark ET Willems¹, Dr Stephen D Myers¹, Dr Fiona Myers³, Dr Nathan A Lewis⁴, Dr Sam D Blacker¹

Affiliations:

¹University of Chichester, Institute of Sport, Chichester, UK

²Galway-Mayo Institute of Technology Galway, Galway, IE

³University of Portsmouth, School of Biological Sciences, Portsmouth, UK

⁴English Institute of Sport, Bath, UK

⁵Oxford Brookes University, Oxford Brookes Centre for Nutrition and Health, Oxford, UK

⁶Coventry University, Centre for Sport, Exercise and Life Sciences, Coventry, UK

⁷Department of Sport, Exercise and Rehabilitation, Northumbria University, Newcastle Upon Tyne, UK

Corresponding author:

Dr Sam Blacker PhD

Institute of Sport,

University of Chichester,

Chichester,

PO19 6PE

United Kingdom

Email: s.blacker@chi.ac.uk

Telephone: +44 (0)1243 793473

Abstract

New Zealand blackcurrant (NZBC) extract is a rich source of anthocyanins and in order to exert physiological effects, the anthocyanin-derived metabolites need to be bioavailable *in vivo*. We examined the plasma uptake of selected phenolic acids following NZBC extract supplementation alongside maintaining a habitual diet (i.e., not restricting habitual polyphenol intake). Twenty healthy volunteers (9 females, age: 28 ± 7 years, height 1.73 ± 0.09 m, body mass 73 ± 11 kg) consumed a 300 mg NZBC extract capsule (CurraNZ™; anthocyanin content 105 mg) following an overnight fast. Venous blood samples were taken pre and 1, 1.5, 2, 3, 4, 5, and 6 h post-ingestion of the capsule. Plasma concentrations of vanillic acid (VA), gallic acid (GA), and protocatechuic acid (PCA) were analysed by reversed-phase high-performance liquid chromatography (HPLC) and the HPLC analysis identified two dihydroxybenzoic (VA and PCA) and one trihydroxybenzoic acids (GA) in plasma following NZBC extract supplementation. Habitual anthocyanin intake was 168 (95%CI:68–404) $\text{mg} \cdot \text{day}^{-1}$ and no associations were observed between this and VA, PCA, and GA plasma uptake by the NZBC extract intake. Plasma time-concentration curves revealed that GA, and PCA were most abundant at 4, and 1.5 h post-ingestion, representing a 261% and 320% increase above baseline, respectively, with VA remaining unchanged. This is the first study to demonstrate that an NZBC extract supplement increases the plasma uptake of phenolic acids GA, and PCA even when a habitual diet is followed in the days preceding the experimental trial, although inter-individual variability is apparent.

Keywords. Anthocyanin; Acute-Supplementation; Phenolic Acids; Systemic Appearance; Habitual Diet

Introduction

Within the last decade, consuming functional foods such as whole berry fruits or concentrated berry extracts has grown in popularity likely due to their reported sport and exercise performance benefits

(Bell, McHugh, Stevenson & Howatson, 2014; Cook & Willems, 2018) attributed to the berry fruits rich polyphenol content (Bowtell & Kelly, 2019). Flavonoids are one of the most abundant naturally occurring polyphenols, which have been the focus of much research interest and can be divided into six subclasses: anthocyanins, flavonols, flavones, flavanones, flavanols and isoflavones (Martin & Appel, 2010).

Blackcurrant is a rich source of anthocyanins; however, the anthocyanin content can vary depending on the berry fruits specific cultivar, cultivation site, temperature exposure, processing, storage and ripeness (Mikulic-Petkovsek, Koron, Zorenc & Veberic, 2017; Del Rio, Borges & Crozier, 2010; Chalker-Scott, 2008). The distinct purple colour of blackcurrants is due to the high levels of anthocyanins, which are glycosides generated from anthocyanidins and are the pigments often responsible also for the orange, red, and blue colours in fruits, vegetables, flowers and other storage tissues in plants (Blando, Gerardi & Nicoletti, 2004). Native cultivars of blackcurrants grown in New Zealand reportedly have a higher anthocyanin content than European cultivars grown in North America (Schrage et al., 2010; Moyer, Hummer, Finn, Frei & Wrolstad, 2002). For example, juice from the New Zealand cultivar, Ben Rua, containing 477 mg·100 mL⁻¹ anthocyanin compared to juice from the non-New Zealand cultivar, Ojebyn, contained 179 mg·100 mL⁻¹ anthocyanin (Schrage et al., 2010; Moyer, Hummer, Finn, Frei & Wrolstad, 2002).

For anthocyanins to be able to exert any benefit *in vivo*, they would need to be bioavailable in sufficient quantities to produce systemic effects (Koli et al., 2010; Toutain & Bousquet-Melou, 2004). Of those anthocyanin pharmacokinetics data available, it has been suggested that the phenolic acids, such as protocatechuic acid (PCA), often appear in systemic circulation in much higher concentrations than that of their parent compounds and that they could be responsible for the associated exercise performance benefits of anthocyanins (Bowtell & Kelly, 2019; Fang, 2014). These phenolic acids provide unique taste, flavour, and health-promoting properties and are found in many fruits and vegetables (Tomas-Barberan & Espin, 2001). Previously, Slimestad and Solheim (2002) identified ~15 different anthocyanins in Nordic-grown blackcurrant and observed that ~98% of the total anthocyanin content are made up of, delphinidin-3-O-glucoside, delphinidin-3-O-rutinoside, cyanidin-3-O-glucoside, and cyanidin-3-O-rutinoside. The phenolic acids, PCA and gallic acid (GA)

are the most abundant degradation products of cyanidin and delphinidin, respectively, the two major parent anthocyanins detected in NZBC whole berry (Slimestad & Solheim, 2002) and concentrate (Matsumoto et al., 2001). Whereas VA is a reported breakdown phenolic acid of peonidin-3-O-rutinoside, which has been identified as a minor compound in blackcurrant (Matsumoto et al., 2001).

The high anthocyanin content within New Zealand blackcurrant (NZBC) has led to an increase in research interest in this particular cultivar. Lyall et al., (2009) first reported acute effects by intake of NZBC extract capsules (240 mg anthocyanins in total) immediately before and following 30 minutes of rowing on an ergometer in 10 healthy males and females (age: 48 ± 2.5 years). The authors concluded that the anthocyanins in NZBC were able to mitigate exercise-induced oxidative stress and dampen the plasma creatine kinase (CK) response, a marker of micro and macro muscle damage, compared to the placebo group 24 h postexercise. Subsequent NZBC extract supplementation studies have utilised a repeated dosing strategy, whereby seven-days before the experimental visit, participants supplemented with NZBC each day, and on day seven, performed the set exercise task/assessment. Supplementation with NZBC extract has been shown to have a range of potential sport and exercise performance benefits, such as increased fat oxidation (Cook, Myers, Blacker & Willems, 2015; Cook, Myers, Gault, Edwards & Willems, 2017a; Şahin, Bilgiç, Montanari & Willems, 2020; Strauss, Willems & Shepherd, 2018), improved cardiovascular response at rest and during exercise (Willems, Myers, Gault & Cook, 2015; Cook, Myers, Gault, Edwards & Willems, 2017b) and enhanced exercise performance (Cook, Myers, Blacker & Willems, 2015; Perkins, Vine, Blacker & Willems, 2015; Murphy et al., 2017; Godwin et al., 2017). These studies have used either commercially available NZBC extract in the form of capsules (CurraNZ™, Health Currancy Ltd, Surrey, UK) or NZBC powder dissolved in water (Sujon New Zealand blackcurrant, Gibb Holdings Ltd, New Zealand) at total anthocyanin doses of $105 \text{ mg} \cdot \text{day}^{-1}$ (Perkins et al., 2015; Cook et al., 2015; Cook et al., 2017a; Cook et al., 2017b), $138.6 \text{ mg} \cdot \text{day}^{-1}$ (Willems et al., 2015; Willems et al., 2017), $210 \text{ mg} \cdot \text{day}^{-1}$ (Cook et al., 2017a; Cook et al., 2017b; Willems et al., 2016; Murphy et al., 2017; Godwin et al., 2017; Strauss, Willems & Shepherd, 2018; Şahin et al. 2020) and $315 \text{ mg} \cdot \text{day}^{-1}$ (Cook et al., 2017a; Cook et al., 2017b). These doses are higher than the reported estimations of habitual anthocyanin dietary intake, which range from 19 to $65 \text{ mg} \cdot \text{day}^{-1}$ in European countries (Zamora-Ros et al., 2011). Interestingly, Cook et al., (2017ab) explored the dose response effects of 7-days intake of NZBC extract

supplementation during exercise and at rest, respectively. Cook et al. (2017b) observed that a dose response effect was apparent for cardiac output and stroke volume between control and 600 and 900 mg·day⁻¹, respectively, in endurance-trained male cyclists during supine rest. In addition, total peripheral resistance decreased by 4±3, 5±9 and 3±4 mmHg·L⁻¹ between control and 600 mg·day⁻¹, control and 900 mg·day⁻¹ and 300 and 900 mg·day⁻¹, respectively. Whereas Cook et al., (2017a) reported dose response effects for both fat and carbohydrate oxidation during 120-minute cycling bouts at 65% $\dot{V}O_{2max}$ in endurance-trained male cyclists. The dose response effect for mean fat oxidation revealed values of 0.63±0.21, 0.70±0.17, 0.73±0.19, and 0.73±0.14 g·min⁻¹ for control, 300, 600 and 900 mg·day⁻¹, respectively, while dose dependent values in mean carbohydrate oxidation were 1.78±0.51, 1.65±0.48, 1.57±0.44, and 1.56±0.50 g·min⁻¹ for control, 300, 600 and 900 mg·day⁻¹, respectively (Cook et al., 2017a). The authors attributed the dose response benefits to a possible build-up of anthocyanin-derived metabolites over the 7-day intake, where previously beneficial vascular responses following anthocyanin intake have been associated with a peak in phenolic metabolites such as vanillic acid (VA) and attributed to the phenolic metabolite's actions on neutrophil nicotinamide adenine dinucleotide phosphate oxidase activity (Rodriguez-Mateos et al., 2013). However, it is important to note that Rodriguez-Mateos et al., (2013) examined the effects of blueberry anthocyanins, which have a different anthocyanin profile to NZBC, and thus may present different plasma metabolite profiles. Furthermore, it is possible that the beneficial effects observed were the result of acute responses to the NZBC extract supplementation, where the last doses were ingested 2 h prior to the commencement of the study measurements (e.g., Şahin et al. 2020).

To date, none of the aforementioned NZBC extract supplementation studies have provided data on the plasma uptake of the phenolic acids of anthocyanins. Understanding the plasma uptake of both acute and repeated intakes of NZBC may help inform optimal dosing strategies and intake guidelines and move dosing protocols towards a more rigorous pharmacokinetic approach. However, most plasma uptake studies incorporate dietary polyphenol restriction in their design in an attempt to reduce the background noise that may be introduced by variation in dietary polyphenol intake (Matsumoto et al., 2001; Keane et al., 2016). This dietary restriction approach may also maximise the effects produced by polyphenol supplementation (Bowtell & Kelly, 2019). Therefore, it has been suggested that to ensure ecological validity, not restricting polyphenol intake in the lead-up to an

experimental trial, would be the most appropriate approach to assessing the plasma uptake of phenolic acids by intake of anthocyanin-rich berries and their potential ability to exert a physiological effect.

Despite a number of studies indicating beneficial effects of NZBC extract supplementation, the plasma uptake of phenolic acids, VA, GA and PCA following NZBC extract ingestion when supplemented whilst consuming a habitual diet has yet to be investigated. Therefore, this investigation aims to examine the time course of VA, GA, and PCA following acute ingestion of a single dose of NZBC extract in individuals following a habitual diet. It was hypothesised that NZBC extract supplementation would increase plasma concentrations of VA, GA, and PCA in the hours following acute ingestion.

Materials and methods

Eleven healthy men and nine women (Table 1) volunteered to participate in the study. All participants were non-smokers, in apparent good health, with no known food allergies, and not currently using any nutritional supplementation. The study was approved by the University's Research Ethics Committee (Protocol Number 1718_02) and conducted in accordance with the Declaration of Helsinki (2013). Participants gave their written informed consent after an explanation of the experimental procedures.

Study design

Participants completed a self-report food diary in the three days leading up to the laboratory visit and refrained from strenuous exercise in the 48 h before their visit. In addition, participants completed a food frequency questionnaire to quantify habitual anthocyanin intake reflective of the past three months (Cook et al., 2017). On arrival to the laboratory after an overnight fast (≥ 12 h), the participant's height and body mass were recorded, and a cannula (BD Venflon™ intravenous cannula with port, 18 G, Becton Dickinson, Wokingham, UK) was inserted into an antecubital vein. Resting venous blood samples were drawn pre and 1, 1.5, 2, 3, 4, 5, and 6 h after ingestion of the NZBC extract capsule with water. Participants remained seated and rested for the duration of the blood sample time points to control for compartmental fluid shifts associated with changes in body posture

(Hagan, Diaz & Horvath, 1978). Participants were permitted to only consume water *ab libitum* during their laboratory visit.

Supplementation, dietary intake and habitual anthocyanin intake

The NZBC extract capsules (CurraNZTM, Health Currancy Ltd, Surrey, UK) were stored at room temperature (~20 °C) in an opaque container before use to avoid UV light degradation (Fossen, Luis & Andersen, 1998). Participants consumed one NZBC extract capsule (300 mg containing 105 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) after the resting blood draw. Participants were instructed to maintain their normal diet before their trial visit to maintain study ecological validity (Bowtell & Kelly, 2019). Participants recorded their three-day dietary intake in food diaries which were analysed (Nutritics LTD, Dublin, Ireland) for carbohydrate, fat and protein, and total energy intake (kJ). The food frequency questionnaire detailed the amount and frequency of anthocyanin-containing foods eaten within the last three months from the Phenol Explorer database (Neveu et al., 2010). The intake of anthocyanin was then calculated as the sum of the consumption frequency of each anthocyanin containing food, multiplied by the content of the anthocyanin content for the portion sizes.

Blood sampling

Fasting whole blood samples were collected into two chilled 5 mL K3 EDTA tubes (Sarstedt, AG and Co, Kommanditgesellschaft, Germany), inverted to mix the anticoagulant and immediately centrifuged at 4100 rpm for 10 min at 4°C. Plasma was aspirated and pipetted into ~1 mL aliquots and then immediately stored at -80°C for subsequent analysis.

Plasma extraction

A method previously described (Keane et al., 2016) was used for the extraction of phenolic compounds from the plasma. Briefly, 1 mL of plasma and 0.5 mL of propyl gallate (internal standard, 50 µg, 100 µL/mL) was mixed with 4 mL oxalic acid (10 nM) and 0.1 mL hydrochloric acid (HCl; 12.6 M) in 15 mL falcon tubes and centrifuged at 3000 rpm for 15 minutes. The supernatant was absorbed on to a primed (washed with 5 mL methanol (MeOH) with 0.2% trifluoroacetic acid (TFA) followed by

2x5 mL of water) solid-phase extraction cartridge (Waters Sep-Pak c17, 360 mg sorbent per cartridge, 55-105 µm). The sample was eluted with 3 mL of MeOH + 0.2% TFA and dried under N₂ at 45°C. Samples were then reconstituted in 400 µl of 0.1% formic acid in water: 2% HCl in MeOH (1:1) and filtered through a 0.2 µm polytetrafluoroethylene filter before HPLC analysis. Samples were analysed on a batch basis, where each batch included standards prepared in 0.1% formic acid in water: 2% HCl in MeOH (1:1), blank control, plasma samples, and fortified plasma samples at 1 (low), 10 (medium), and 25 (high) µg/mL. The recovery ranges were 89-95%, 89-102%, and 90-103% for low, medium, and high fortified levels, respectively. The final results were collected for recovery at the low fortification level.

High-Performance Liquid Chromatography (HPLC) analysis

A high-pressure liquid chromatography-diode array detector (DAD) method for the detection and quantitation of selected phenolic compounds in the plasma samples (pre-supplementation through to 6 h post-supplementation) was carried out using a Dionex UltiMate 3000 HPLC System (Dionex, Camberley, UK) equipped with an UltiMate 3000 RS pump, an UltiMate 3000 autosampler, and a 3000 RS UV/Vis Detector and a RS Fluorescence Detector (FLD). The filtered samples (20 µL) were injected on a Phenomenex Luna C₁₈(2) (250x2.0 mm, 5 µm particle size) reverse-phase column thermostat-controlled at 30°C. The mobile phase consisted of water with 1% acetic acid (solvent A), and acetonitrile with 1% acetic acid (solvent B). After a 5-minute equilibration with 20% A, the elution programme was as follows: 0-15 min, 20-100% B, (0.2 mL/min) followed by a washing stage (100% B, 15-18 min, 1.0 mL/min) and return at the initial conditions within 2 minutes. Detection was performed at the following excitation/emission wavelengths: λ_{ex} = 278 nm and λ_{em} = 360 nm for PCA and propyl gallate (PG), λ_{ex} = 278 nm and λ_{em} = 366 for GA and λ_{ex} = 260 nm and λ_{em} = 422 nm for VA, respectively. The identification and quantitation of PCA, GA, and VA content of plasma samples was based on a combination of retention time and spectral matching of reference standards. Final results are expressed as micrograms per millilitre (µg/mL).

Statistical analysis

Statistical analyses were completed using Statistical Package for Social Sciences 23.0 (SPSS, Chicago, Illinois). All dependent variables were analysed using a treatment (300 mg NZBC dose) by

time (0, 1, 1.5, 2, 3, 4, 5, and 6 h) one-way, repeated-measures analysis of variance (ANOVA). Mauchly's test of sphericity was used to check homogeneity of variance for all variables; where necessary, any violations of the assumption were corrected using the Greenhouse-Geisser adjustment. Main effects for time were followed up using Bonferroni *post hoc* analysis. Further analysis was conducted to identify maximum **group and mean of each individual** plasma concentrations (C_{max}) and times to achieve maximum plasma concentrations (t_{max}), which were directly obtained from the plasma concentration-time profiles (Toutain & Bousquet-Melou, 2004). As a measure of overall plasma uptake of individual phenolic acids, the area under the plasma concentration-time curve (AUC_{0-6h}) for each participant was estimated by using the linear trapezoidal rule, with the total sum and mean of each individual being reported. **Pearson (r) correlation coefficients were calculated for the relationship between habitual anthocyanin intake and total sum AUC_{0-6h} for each metabolite (VA, GA, and PCA) and between each metabolite total sum AUC_{0-6h} (VA, GA, and PCA).** Pearson (r) correlation coefficients of <0.3, <0.5 and ≥ 0.5 are interpreted as weak, moderate and strong correlations, respectively (Cohen, 1988). The alpha level for statistical significance was set at 0.05 *a priori* and Partial-eta² (η^2) effect sizes (ANOVA) are reported to indicate the magnitude of observed effects (Lakens, 2013). Partial-eta² (η^2) effect sizes of 0.01–0.06, 0.06–0.14 or ≥ 0.14 are considered small, medium and large changes, respectively (Lakens, 2013). Data in text and tables are reported as mean (95% confidence intervals) and data in figures as mean with individual data points (Weissgerber, Milic, Winham & Garovic, 2015).

Results

Vanillic acid (VA) results are based on $n=17$ and PCA on $n=18$ participants', due to their values falling below the LOD during HPLC analysis. Gallic acid results are based on $n=20$. Table 1 reports data for the 72-h food diaries and anthocyanin food frequency questionnaire.

Please insert **Table 1** near here

Plasma time-concentration curves

Vanillic acid (VA)

There was no main effect of time on plasma VA plasma concentration ($F=2.218$, $p=0.109$, $\eta^2=0.12$, η^2 95%CI: 0–0.28; **Figure 1a**). Table 2 shows the t_{\max} , C_{\max} , total AUC_{0-6h} and the mean of each individual AUC_{0-6h} for VA.

Gallic acid (GA)

There was a main effect of time on plasma GA concentration ($F=25.273$, $p=0.001$, $\eta^2=0.57$, η^2 95%CI: 0.40–0.66). Pairwise comparisons revealed that GA concentrations were increased at 1, 1.5, 2, 3, 4, 5 and 6 h following supplementation when compared to 0 h ($p<0.0001$; **Figure 1b**). Furthermore, plasma GA concentrations were elevated at 3, 4, 5 and 6 h compared to 1 h following supplementation ($p<0.05$). GA levels in plasma were higher 4 and 5 h compared to 1.5 h following supplementation ($p=0.004$ and $p=0.011$, respectively). Table 2 shows the t_{\max} , C_{\max} , total AUC_{0-6h} and the mean of each individual AUC_{0-6h} for GA.

Protocatechuic acid (PCA)

Following supplementation, there was a main effect of time on PCA plasma levels ($F=10.638$, $p=0.001$, $\eta^2=0.36$, η^2 95%CI: 0.11–0.52). Pairwise comparisons revealed that PCA concentrations were elevated at 1, 1.5, 2, 3, 4, 5 and 6 h following supplementation when compared to 0 h ($p<0.05$ **Figure 1c**). Table 2 shows the t_{\max} , C_{\max} , total AUC_{0-6h} and the mean of each individual AUC_{0-6h} for PCA.

Please insert **Figure 1a, 1b and 1c** near here

Please insert **Table 2** near here

Relationships between phenolic compounds and habitual anthocyanin intake

There were no significant correlations between estimated habitual anthocyanin intake and the total AUC_{0-6h} for VA, or PCA (**Table 3**). Table 3 shows that a moderate positive significant correlation was observed between VA and PCA. However, no significant correlations were observed for GA and PCA and GA and VA.

Please insert **Table 3** near here

Discussion

This is the first study to demonstrate that a NZBC extract supplement increases the plasma uptake of phenolic acids GA, and PCA even when a habitual diet is followed in the days preceding the experimental trial (i.e., no restriction of polyphenol intake). The novel approach to this study was to investigate the appearance and 6 h time course of these phenolic acids in plasma following acute consumption of a NZBC extract supplement.

There is a limited understanding about the metabolism and absorption of VA, GA, and PCA following NZBC intake where only one pilot study has explored the plasma phenolic acid appearance following blackcurrant extract ingestion **with participants on an anthocyanin-free diet 72 hrs before** (Roehrig, Kirsch, Schipp, Galan & Richling, 2019). **The** present study demonstrated that PCA and GA are most bioavailable in plasma 1.5 and 4 h post-NZBC extract ingestion, respectively **(Table 2)**. An independent HPLC analysis on a batch of the NZBC extract capsules confirmed no presence of peonidin-3-O-rutinoside. However, in our study, the VA (a downstream metabolite of peonidin-3-O-rutinoside) was detectable in the plasma in 17 of the 20 participants, with transient increases from baseline observed in four out of 20 participants **(Figure 1a)**, **despite no group main effects of time being apparent**. The transient increases in VA response were not uniformly observed within the cohort. As such we cannot determine if any change in this metabolite is a result of NZBC extract supplementation *per se* or an interaction between a dietary feature shared between these four participants, but absent from the others, or simply a noise/measurement artifact. **Peonidin-3-O-rutinoside** has been shown to be a minor anthocyanin compound in blackcurrant (Frøytlog, Slimestad, & Andersen, 1998). The phenolic acid PCA, **however**, can be extensively metabolized to numerous metabolites such as VA, hippuric acid, ferulic acid, and 4-hydroxybenzaldehyde (Gao et al., 2006; de Ferrars et al., 2014). Therefore, it is possible that presence of VA in plasma over the 6 h was due to enterohepatic metabolism of PCA to VA. Thus, possibly being the reason as to why a positive relationship was observed in this present study between VA and PCA. However, it must be acknowledged that, **despite participants completing an overnight fast before consuming the NZBC supplement**, habitual dietary intake leading up to the experimental visit, could also be a contributing

factor to the appearance of VA in plasma. High concentrations of VA *in vivo* have been linked to the abundance of anthocyanins in fruits and vegetables (Nurmi et al., 2009). Vanillic acid, GA, and PCA were present at baseline in plasma for all participants before NZBC extract consumption, which is in accordance with previous research, where anthocyanin metabolites were still observable 48 h post-cyanidin 3-O-glucoside bolus ingestion (Czank et al., 2013). Thus, it is plausible that foods rich in anthocyanins consumed by the participants before the 12 h overnight fast, resulted in bioaccumulation of phenolic acids, which were then detectable in plasma on the morning of the laboratory visit. Despite the equivocal results for this metabolite, future research would benefit from retaining VA within the panel of metabolites measured to shed further light on the interindividual variability.

Slimestad and Solheim (2002) previously highlighted that **the main pigments from whole blackcurrant berries are delphinidin and cyanidin and that these structures constitute ~97% of the extractable anthocyanin content from these blackcurrant berries**. Post-NZBC extract ingestion, GA increased in plasma concentration across the 6 h time frame, achieving group t_{\max} by 4 h, presented the greatest plasma uptake and had not returned to baseline at 6 h post-ingestion (**Figure 1b**). This finding is in contrast to previous work, which reported GA t_{\max} occurred at 1 h post-ingestion of a blackcurrant extract (Roehrig et al., 2019), however, this result was based on one participant. Gallic acid is a trihydroxybenzoic acid and the major stable phenolic acid derived from ring fission of the delphinidin skeleton, which is the most abundant anthocyanin in NZBC. A study that investigated the enzymatic potential for *Bifidobacteria* and *Lactobacillus*, two predominant members of the intestinal microflora, to convert delphinidin and malvidin glycosides into their phenolic acid degradation compounds, observed that the *Lactobacillus* strain, *L. casei*, resulted in the highest concentrations of GA after 24 h of incubation (Ávila et al., 2009). Furthermore, the authors highlighted that as delphinidin 3-O-glucoside underwent chemical degradation to form mainly GA, and concentrations of this phenolic acid were detected at 1 h in samples, which increased throughout the time period concomitantly with delphinidin 3-O-glucoside clearance. Therefore, it is feasible that the appearance of GA in plasma over the 6 h in the present study was possibly a result of the gut microbiome profiles of the participants. Future research should consider studying faecal microflora samples before and following an anthocyanin-rich

berry fruit supplementation to assess this relationship further (Tomas-Barberan, Selma & Espin, 2018).

Protocatechuic acid is the main degradation compound of cyanidin (Vitaglione et al., 2007). Previous research has reported observing a biphasic response in the serum of PCA metabolite kinetics, displaying an initial peak between 0 and 5 h and a second peak between 6 and 48 h following an isotopically labelled bolus of cyanidin 3-O-glucoside (de Ferrars et al., 2014). A similar response was apparent for several participants in the present study, where an initial peak occurred around 1 h, followed by a second peak at 3 h (**Figure 1c**). The reason for these biphasic profiles has been suggested to be a result of metabolism occurring in multiple tissues, such as the liver, and at different sites within the gastrointestinal tract (de Ferrars et al., 2014). Following blood orange consumption Vitaglione et al., (2007) observed a recovery of cyanidin 3-O-glucoside and PCA in the 24 h faecal samples, which they suggested indicated *in vivo* production of PCA by intestinal microflora, and that this slow and continuous release of antioxidant compounds into systemic circulation may have physiological relevance to maintain the concentration of blood antioxidants over 24 h. With regards to tissue accumulation of anthocyanin, Kirakosyan et al., (2015), studied the effect of 3-weeks of anthocyanin-rich cherry supplementation in rats on tissue bioavailability and found that some tissues preferentially stored phenolic acids. However, when humans supplemented over a 12-week period with anthocyanin-rich elderberry extract, no differences were observed in the concentration of metabolites post-prandial in urine before and following the 12-weeks of repeated anthocyanin dosing (de Ferrars, Cassidy, Curtis & Kay, 2013). Therefore, further research is warranted on whether anthocyanin metabolites can be preferentially stored in tissue or whether bioaccumulation is limited to a short window of opportunity.

The individual participant uptake in plasma of VA, GA and PCA (**Figure 1a, b, c**) observed in the present study, supports previous observations by Keane et al. (2016) where large inter-individual variability was apparent following acute intake of 30 and 60 mL of Montmorency tart cherry concentrate. In an attempt to quantify the variation in individual participant plasma uptake in the present study, the mean of each individual AUC_{0-6h} plasma time-concentration curves (linear trapezoidal model), *t_{max}* and *C_{max}* were estimated. Comparing the mean of each individual AUC_{0-6h}, *t_{max}*

and C_{\max} responses to the mean total AUC_{0-6h} , t_{\max} and C_{\max} responses, it is apparent that some individuals present greater plasma uptake for each metabolite than others. This could explain why in some NZBC extract supplementation studies, the magnitude of the response (i.e. fat oxidation) to the intervention varies between participants (Cook et al., 2015; Cook et al., 2017; Strauss et al., 2018). Large inter-individual variations in the plasma concentrations of phenolic compounds following an 8-week mixed berry diet intervention have been observed previously (Koli et al., 2010), with these variations being attributed to differences within participant's intestinal microflora, which can be affected by dietary intake and genetic differences. However, no relationships were observed in the present study between estimated habitual anthocyanin intake and overall VA, GA, and PCA plasma uptake (quantified by total AUC_{0-6h}).

Given the current focus on individualised sport and exercise nutrition (Betts & Gonzalez, 2016), it appears that when considering using an anthocyanin-rich supplement, such as NZBC, practitioners should take into consideration the large inter-individual response that can occur. For example, if taking the mean response from this current study, it would seem plausible to suggest that most individuals will reach their peak plasma concentration for PCA at ~1.5 h, however, as can be observed in **Figure 1c and Table 2**, several participants did not achieve their peak plasma concentration until ~3 h. Future research should consider quantifying the plasma uptake of anthocyanin metabolites before and following berry fruit extract supplementation, alongside physiological performance measures to assess the relationship between them as has been suggested previously (Bowtell & Kelly, 2019).

The main limitation of the present study is the lack of a control group or condition; thus, we cannot infer whether any of the changes in the metabolites are due to the NZBC supplementation, or are natural fluctuations observed as a response of circadian variation over the 6 h. However, the changes in metabolites being due to natural fluctuations are highly unlikely, given the magnitude of change and that the participants had completed an overnight fast before the supplement was consumed. Additionally, the use of the 0 h timepoint does provide a control baseline of plasma VA, GA and PCA prior to the NZBC extract being ingested for comparative purposes. Furthermore, the analysis in the present study was not exhaustive and so no parent anthocyanins were analysed nor was every

plasma metabolite was analysed; instead, the focus was on the degradation products of two of the main anthocyanins reported in NZBC extract and VA, the methylated degradation compound of PCA, that have previously been shown to exert positive effects on vascular function (Keane et al, 2016).

Given that anthocyanins degrade and are extensively metabolised *in vivo*, it is possible that accumulation of multiple phenolic metabolites may ultimately be responsible for the reported bioactivity of anthocyanins (Kirakosyan et al., 2015). In addition, the present study utilised an acute dose of NZBC extract (105 mg anthocyanin) and so it is not possible to forecast the impact of a longer and/or repeated supplementation period on plasma phenolic uptake. Although previous NZBC research has utilised longer (7-day) supplementation periods, none have quantified the plasma uptake of anthocyanin phenolic aids such as VA, GA and PCA. For the first time, this study characterizes the appearance of VA, GA and PCA following an acute dose of NZBC extract. Future research is warranted to establish whether a repeated dose can provide additional physiological benefits with regards to plasma uptake or whether a ceiling effect exists in light of the dose response benefits observed by Cook et al., (2017a) and (2017b). It would also be of interest to examine the plasma uptake of phenolic acids with repeated intakes of NZBC extract during the day alongside normal food intake. Lastly, the timeframe of this current investigation lasted until 6 h, with VA, GA and PCA plasma levels still elevated at this time point. Previous studies focusing on the degradation products of cyanidin have reported a presence of VA in serum at ~42 h post isotopically labelled cyanidin 3-O-glucoside bolus ingestion (Czank et al., 2013) and so, it is probable that the phenolic acids in this study could have remained present in plasma until ~42 h. Future research should incorporate a more extensive panel of parent anthocyanins along with possible gut microbial metabolites, utilise a control group and extend the study timeframe up to 48 h as has been done in previous pharmacokinetic studies (Czank et al., 2013) in order to be able to draw firm conclusions.

Nevertheless, this study presents new information regarding the presence *in vivo* of anthocyanin phenolic acids, VA, GA, and PCA from acute NZBC extract supplementation when a habitual diet is followed in the days preceding the experimental trial. The time course of phenolic acid accumulation peaks between 1.5 and 4 h post-NZBC extract ingestion depending on the phenolic acid of interest; however, large inter-individual variability is apparent. This information could inform future *in vivo* work that examines the sport and exercise related benefits with acute NZBC extract supplementation by

indicating the times at which peak plasma concentrations are likely to occur and the overall plasma uptake of VA, GA, and PCA. This could in turn be used to suggest appropriate timings of supplementation before exercise intervention onset when a habitual diet is followed.

Acknowledgements

The authors would like to thank their participants in this investigation and the late Miss Ania Hiles for her assistance in collecting participant blood samples.

Declaration of interest

Health Currancy (United Kingdom) Ltd and CurraNZ (New Zealand) Ltd provided supplementation. However, Health Currancy (United Kingdom) Ltd and CurraNZ (New Zealand) Ltd had no role in any aspect of the study and manuscript. The authors report no conflicts of interest.

References

- Ávila, M., Hidalgo, M., Sanchez-Moreno, C., Pelaez, C., Requena, T., & de Pascual, T.S. (2009). Bioconversion of anthocyanin glycosides by *bifidobacteria* and *lactobacillus*. *Food Research International*, 42: 1453-1461.
- Bell, P.G., McHugh, M.P., Stevenson, E., & Howatson, G. (2014). The role of cherries in exercise and health. *Scandinavian Journal of Medicine & Science in Sports*, 24(3): 477-490. doi:10.1111/sms.12085
- Betts, J.A., & Gonzalez, J.T. (2016). Personalised nutrition: what makes you so special? *British Nutrition Foundation Bulletin*, 41: 353-359.
- Blando, F., Gerardi, C., & Nicoletti, I. (2004). Sour cherry (*Prunus cerasus* L) anthocyanins as ingredients for functional foods. *Journal of Biomedicine & Biotechnology*, 5: 253-258. doi:10.1155/s1110724304404136.
- Bowtell, J., & Kelly, V. (2019). Fruit-Derived Polyphenol Supplementation for Athlete Recovery and Performance. *Sports Medicine*, 49(1): 3-23. doi:10.1007/s40279-018-0998-x.
- Chalker-Scott, L. (2008). Environmental significance of anthocyanins in plant stress responses. *Photochemistry and photobiology*, 70(1): 1-9.

Cohen, J. (1988). Statistical power analysis for the behavioural sciences (2nd ed). Hillsdale, NJ: Lawrence Erlbaum Associates.

Cook, M.D., & Willems, M.E.T. (2018). Dietary Anthocyanins: A review of the exercise performance effects and related physiological responses. *International Journal of Sports Nutrition & Exercise Metabolism*, 29(3): 1-9. doi:10.1123/ijsnem.2018-0088.

Cook, M.D., Myers, S.D., Blacker, S.D., & Willems, M.E.T. (2015). New Zealand Blackcurrant extract improves cycling performance and fat oxidation in cyclists. *European Journal of Applied Physiology*, 115(11): 2357-2365.

Cook, M.D., Myers, S.D., Gault, M.L., Edwards, V.C., & Willems, M.E.T. (2017a). Dose effects of New Zealand blackcurrant on substrate oxidation and physiological responses during prolonged cycling. *European Journal of Applied Physiology*, 117(6): 1207-1216. doi:10.1007/s00421-017-3607-z.

Cook, M.D., Myers, S.D., Gault, M.L., Edwards, V.C., & Willems, M.E.T. (2017b). Cardiovascular function during supine rest in endurance-trained males with New Zealand blackcurrant: a dose-response study. *European Journal of Applied Physiology*, 117(2): 247-254. doi:10.1007/s00421-016-3512-x.

Czank, C., Cassidy, A., Zhang, Q., Morrison, D.J., Preston, T., Kroon, P.A., . . . & Kay, C.D. (2013). Human metabolism and elimination of the anthocyanin, cyanidin-3-glucoside: a (13)C-tracer study. *American Journal of Clinical Nutrition*, 97(5): 995-1003. doi:10.3945/ajcn.112.049247.

de Ferrars, R.M., Cassidy, A., Curtis, P., & Kay, C.D. (2013). Phenolic metabolites of anthocyanins following a dietary intervention study in post-menopausal women. *Molecular Nutrition & Food Research*, 58(3): 490-502. doi:10.1002/mnfr.201300322.

de Ferrars, R.M., Czank, C., Zhang, Q., Botting, N.P., Kroon, P.A., Cassidy, A., & Kay, C.D. (2014). The pharmacokinetics of anthocyanins and their metabolites in humans. *British Journal of Pharmacology*, 171(13): 3268-3282. doi:10.1111/bph.12676.

Del Rio, D., Borges, G., & Crozier, A. (2010). Berry flavonoids and phenolics: bioavailability and evidence of protective effects. *British Journal of Nutrition*, 104(3): 67-90. doi:10.1017/s0007114510003958.

Fang, J. (2014). Bioavailability of anthocyanins. *Drug Metabolism Reviews*, 46(4): 508-520. doi:10.3109/03602532.2014.978080.

- Fossen, T., Luis, C., & Andersen, Ø.M. (1998). Colour and stability of pre anthocyanins influenced by pH including the alkaline region. *Food Chemistry*, 63(4): 5.
- Frøytlog, C., Slimestad, R., & Andersen, Ø.M. (1998). Combination of chromatographic techniques for the preparative isolation of anthocyanins - applied on blackcurrant (*Ribes nigrum*) fruits. *Journal of Chromatography*. 82: 89-95.
- Gao, K., Xu, A., Krul, C., Venema, K., Liu, Y., Niu, Y., . . . & Henning, S.M. (2006). Of the major phenolic acids formed during human microbial fermentation of tea, citrus, and soy flavonoid supplements, only 3,4-dihydroxyphenylacetic acid has antiproliferative activity. *Journal of Nutrition*, 136(1): 52-57. doi:10.1093/jn/136.1.52.
- Godwin, C., Cook, M.D., & Willems, M.E.T. (2017). Effect of New Zealand blackcurrant extract on performance during the running based anaerobic sprint test in trained youth and recreationally active male football players. *Sports*, 5(3): 69. doi:10.3390/sports5030069.
- Hagan, R.D., Diaz, F.J., Horvath, S.M. (1978). Plasma volume changes with movement to supine and standing positions. *Journal of Applied Physiology: Respiratory, Environmental & Exercise Physiology*, 45(3): 414-417. doi:10.1152/jappl.1978.45.3.41.
- Keane, K.M., Bell, P.G., Lodge, J.K., Constantinou, C.L., Jenkinson, S.E., Bass, R., & Howatson, G. (2016). Phytochemical uptake following human consumption of Montmorency tart cherry (L. *Prunus cerasus*) and influence of phenolic acids on vascular smooth muscle cells in vitro. *European Journal of Nutrition*, 55(4): 1695-1705. doi:10.1007/s00394-015-0988-9.
- Koli, R., Erlund, I., Jula, A., Marniemi, J., Mattila, P., Alfthan, G. (2010). Bioavailability of various polyphenols from a diet containing moderate amounts of berries. *Journal of Agriculture & Food Chemistry*, 58(7): 3927-3932. doi:10.1021/jf9024823.
- Kirakosyan, A., Seymour, E.M., Wolforth, J., McNish, R., Kaufman, P.B., & Bolling, S.F. (2015). Tissue bioavailability of anthocyanins from whole tart cherry in healthy rats. *Food Chemistry*, 171: 26-31. doi:10.1016/j.foodchem.2014.08.114.
- Lakens, D. (2013). Calculating and reporting effect sizes to facilitate cumulative science: a practical primer for t-tests and ANOVAs. *Frontiers in Psychology*, 4: 863. doi:10.3389/fpsyg.2013.00863.
- Lyall, K.A., Hurst, S.M., Cooney, J., Jensen, D., Lo, K., Hurst, R.D., & Stevenson, L.M. (2009). Short-term blackcurrant extract consumption modulates exercise-induced oxidative stress and

lipopolysaccharide-stimulated inflammatory responses. *American Journal of Physiology: Regulatory, Integrative & Comparative Physiology*, 297(1): 70-81. doi:10.1152/ajpregu.90740.2008.

Martin, K.R., & Appel, C.L. (2010). Polyphenols as dietary supplements: A double-edged sword. *Nutrition and Dietary Supplements*, 2: 1-12.

Matsumoto, H., Takenami, E., Iwasaki-Kurashige, K., Osada, T., Katsumura, T., & Hamaoka, T. (2005). Effects of blackcurrant anthocyanin intake on peripheral muscle circulation during typing work in humans. *European Journal of Applied Physiology*, 94(1-2): 36-45. doi:10.1007/s00421-004-1279-y.

Matsumoto, H., Inaba, H., Kishi, M., Tominaga, S., Hirayama, M., & Tsuda, T. (2001). Orally administered delphinidin 3-rutinoside and cyanidin 3-rutinoside are directly absorbed in rats and humans and appear in the blood as the intact forms. *Journal of Agriculture & Food Chemistry*, 49(3): 1546-1551.

Mikulic-Petkovsek, M., Koron, D., Zorenc, Z., & Veberic, R. (2017) Do optimally ripe blackberries contain the highest levels of metabolites? *Food Chemistry*, 215: 41-49. doi:10.1016/j.foodchem.2016.07.144.

Moyer, R.A., Hummer, K.E., Finn, C.E., Frei, B., & Wrolstad, R.E. (2002). Anthocyanins, phenolics, and antioxidant capacity in diverse small fruits: vaccinium, rubus, and ribes. *Journal of Agriculture & Food Chemistry*, 50(3): 519-525.

Murphy, C., Cook, M.D., & Willems, M.E.T. (2017). Effect of New Zealand blackcurrant extract on repeated cycling time trial performance. *Sports*, 5(2): 25. doi:10.3390/sports5020025.

Neveu, V., Perez-Jimenez, J., Vos, F., Crespy, V., du Chaffaut, L., Mennen, L., . . . & Scalbert, A. (2010). Phenol-Explorer: an online comprehensive database on polyphenol contents in foods. Database (Oxford), 2010, bap024. doi:10.1093/database/bap024.

Nurmi, T., Mursu, J., Heinonen, M., Nurmi, A., Hiltunen, R., & Voutilainen, S. (2009). Metabolism of berry anthocyanins to phenolic acids in humans. *Journal of Agriculture & Food Chemistry*, 57(6): 2274-2281. doi:10.1021/jf8035116.

Perkins, I.C., Vine, S.A., Blacker, S.D., & Willems, M.E.T. (2015). New Zealand Blackcurrant extract improves high-intensity intermittent running. *International Journal of Sports Nutrition & Exercise Metabolism*, 25(5): 487-493.

Rodriguez-Mateos, A., Rendeiro, C., Bergillos-Meca, T., Tabatabaee, S., George, T.W., Heiss, C., Spencer, J.P. (2013). Intake and time dependence of blueberry flavonoid-induced improvements in

vascular function: a randomized, controlled, double-blind, crossover intervention study with mechanistic insights into biological activity. *American Journal of Clinical Nutrition*, 98(5): 1179-1191. doi: 10.3945/ajcn.113.066639.

Roehrig, T., Kirsch, V., Schipp, D., Galan, J., & Richling, E. (2019). Absorption of anthocyanin rutinosides after consumption of a blackcurrant (*Ribes nigrum* L.) extract. *Journal of Agriculture & Food Chemistry*. doi:10.1021/acs.jafc.9b01567.

Şahin, M. A., Bilgiç, P., Montanari, S., & Willems, M. E. T. (2020). Intake duration of anthocyanin-rich New Zealand blackcurrant extract affects metabolic responses during moderate intensity walking exercise in adult males. *Journal of Dietary Supplements*, 0(0), 1–12. <https://doi.org/10.1080/19390211.2020.1783421>

Schrage, B., Stevenson, D., Wells, R.W., Lyall, K., Holmes, S., Deng, D., & Hurst, R.D. (2010). Evaluating the health benefits of fruits for physical fitness: A research platform. *Journal of Berry Research*, 1: 35-44.

Slimestad, R., & Solheim, H. (2002). Anthocyanins from blackcurrants (*Ribes nigrum* L.). *Journal of Agriculture & Food Chemistry*, 50: 3228-3231.

Strauss, J.A., Willems, M.E.T., & Shepherd, S.O. (2018). New Zealand blackcurrant extract enhances fat oxidation during prolonged cycling in endurance-trained females. *European Journal of Applied Physiology*, 118(6): 1265-1272. doi:10.1007/s00421-018-3858-3.

Tomas-Barberan, F., & Espin, J.C. (2001). Phenolic compounds and related enzymes as determinants of quality in fruits and vegetables. *Journal of Science of Food & Agriculture*, 81(9):853-876. doi: 10.1002/jsfa.885.

Tomas-Barberan, F.A., Selma, M.V., & Espin, J.C. (2018). Polyphenols' gut microbiota metabolites: bioactives or biomarkers? *Journal of Agriculture & Food Chemistry*. doi:10.1021/acs.jafc.8b00827.

Toutain, P.L., & Bousquet-Melou, A. (2004). Bioavailability and its assessment. *Journal of Veterinary Pharmacology & Therapeutics*, 27(6): 455-466. doi:10.1111/j.1365-2885.2004.00604.x.

Vitaglione, P., Donnarumma, G., Napolitano, A., Galvano, F., Gallo, A., Scalfi, L., & Fogliano, V. (2007). Protocatechuic acid is the major human metabolite of cyanidin-glucosides. *Journal of Nutrition*, 137(9): 2043-2048. doi:10.1093/jn/137.9.2043.

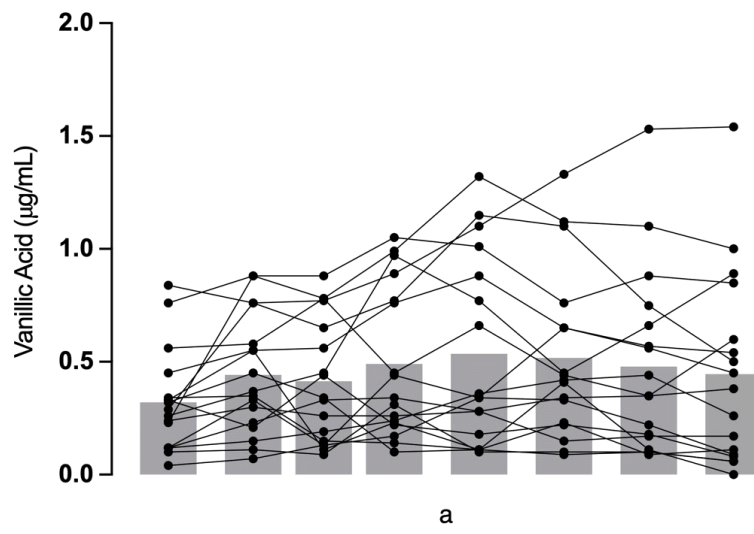
Weissgerber, T.L., Milic, N.M., Winham, S.J., & Garovic, V.D. (2015). Beyond bar and line graphs: time for a new data presentation paradigm. *PLoS Biology*, 13(4): e1002128. doi:10.1371/journal.pbio.1002128.

Willems, M.E.T, Cousins, L., Williams, D., & Blacker, S.D. (2016). Beneficial effects of New Zealand blackcurrant extract on maximal sprint speed during the loughborough intermittent shuttle test. *Sports*, 4(3): 42. doi:10.3390/sports4030042.

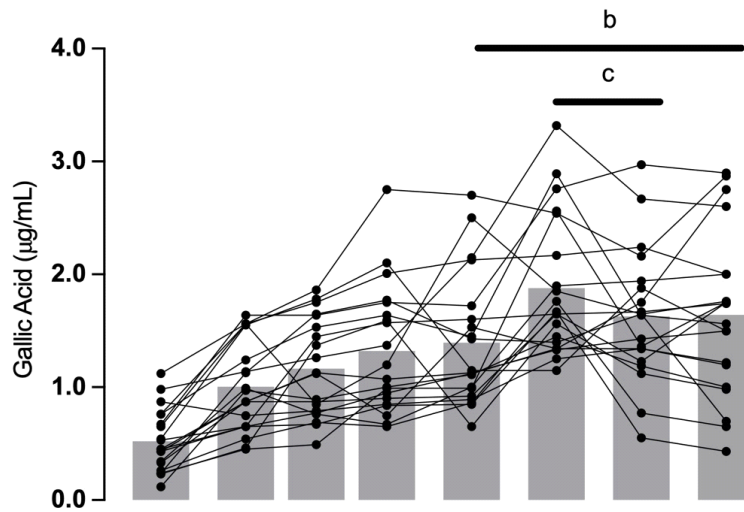
Willems, M.E.T., Myers, S.D., Gault, M.L., & Cook, M.D. (2015). Beneficial physiological effects with blackcurrant intake in endurance athletes. *International Journal of Sports Nutrition & Exercise Metabolism*, 25(4): 367-374. doi:10.1123/ijsnem.2014-0233.

Zamora-Ros, R., Knaze, V., Lujan-Barroso, L., Slimani, N., Romieu, I., Fedirko, V., . . . & Gonzalez, C. A. (2011). Estimated dietary intakes of flavonols, flavanones and flavones in the European Prospective Investigation into Cancer and Nutrition (EPIC) 24 hour dietary recall cohort. *British Journal of Nutrition*, 106(12): 1915-1925. doi:10.1017/s000711451100239x.

1a



1b



1c

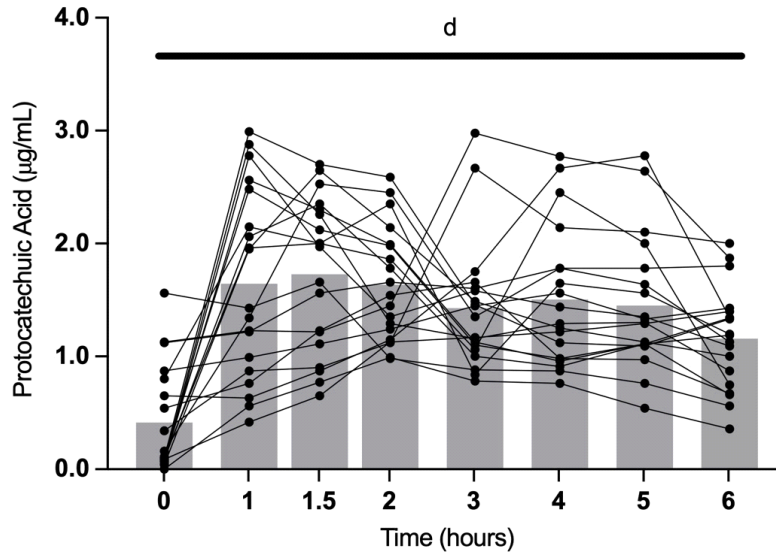


Figure legend

Figure 1 Vanillic acid (**a, n=17**), gallic acid (**b, n=20**) and protocatechuic acid (**c, n=18**) responses from baseline to supplementation with 300 mg New Zealand Blackcurrant extract capsule. Absolute baseline values were 0.27 (95%CI: 0.13-0.68) µg/mL, 0.52 (95%CI: 0.12-0.92) µg/mL and 0.39

(95%CI: 0.40-1.19) µg/mL for VA, GA and PCA, respectively. ^a indicates different from 0 h at all time points (p<0.0001), ^b indicates different between 1 h and 3, 4, 5 and 6 h (p<0.05), ^c indicates different between 1.5 h and 4 and 5 h (p<0.05), ^d indicates different from 0 h at all time points (p<0.05).

Table 1 Mean (95%CI) descriptive data of the participants, absolute energy and macronutrient intake per day 72 h prior to the second experimental visit and habitual anthocyanin intake

Variable	Value
Participant characteristics	
Age (years)	28 (16-41)
Height (m)	1.73 (1.58-1.88)
Body mass (kg)	73 (55-92)
Body mass index (kg·m ⁻²)	24 (20-29)
Nutritional status	
Total energy intake (kJ)	8175 (6034-10317)
Carbohydrate (g)	205 (118-292)
Fat (g)	75 (49-102)
Protein (g)	100 (49-150)
Habitual anthocyanin intake (mg·day ⁻¹)	168 (68-404)

n=20 (11 males).

Table 2. Mean (95%CI) vanillic acid, gallic acid and protocatechuic acid group and individual responses following intake of 300 mg of New Zealand blackcurrant extract.

Phenolic acid	Group T_{\max} (Time point following supplementation)	Individual mean T_{\max} (Time point following supplementation)	Group C_{\max} ($\mu\text{g mL}^{-1}$)	Individual mean C_{\max} ($\mu\text{g mL}^{-1}$)	Total $\text{AUC}_{0-6\text{h}}$ ($\mu\text{g h mL}^{-1}$)	Individual mean $\text{AUC}_{0-6\text{h}}$ ($\mu\text{g h mL}^{-1}$)
Vanillic acid	3 h	3 h (95%CI: 0-5 h)	0.49 (95%CI: 0.21-1.19)	0.71 (95%CI: 0.08-1.35)	48.0 (95%CI: 44.8–51.2)	2.8 (95%CI: 0.4–6.0)
Gallic acid	4 h	4 h (95%CI: 2-7 h)	1.88 (95%CI: 1.24-2.52)	2.12 (95%CI: 1.15-3.08)	166.2 (95%CI: 161.9-170.4)	8.3 (95%CI: 4.0-12.6)
Protocatechuic acid	1.5 h	3 h (95%CI: 0-5 h)	1.66 (95%CI: 0.43-2.89)	2.22 (95%CI: 1.13-3.30)	161.3 (95%CI: 158.0-164.6)	8.5 (95%CI: 5.2-11.8)

t_{\max} , time to maximum concentration; C_{\max} , concentration maximum; $\text{AUC}_{0-6\text{h}}$, area under the curve 0 – 6 h.

Table 3 Pearson's correlation coefficient matrix of habitual anthocyanin intake, total $\text{AUC}_{0-6\text{h}}$ vanillic acid, gallic acid and protocatechuic acid following intake of 300 mg of New Zealand blackcurrant extract.

	Habitual anthocyanin intake	Vanillic Acid	Gallic Acid	Protocatechuic Acid
Habitual anthocyanin intake		-0.107 0.681	-0.059 0.811	0.315 0.189
Vanillic Acid	-0.107 0.681		-0.396 0.129	0.599* 0.014
Gallic Acid	-0.059 0.811	-0.396 0.129		-0.240 0.322
Protocatechuic Acid	0.315 0.189	0.599* 0.014	-0.240 0.322	

Asterisk indicates statistically significant relationships between total AUC_{0-6h} between each metabolite.

Supplementary info

Selected UV-Vis wavelengths and retention times (mins) for quantification of phenolics by HPLC –
UV/Vis

Compound	UV/Vis wavelength (nm)	Retention time (mins)	LOD (µg/mL)	Range of linearity (µg/mL)
PCA	278/360	7.124	<0.04	0.1–50
VA	260/422	9.090	<0.04	0.1–50
GA	278/366	7.732	<0.05	0.1–50
PG	278/360	11.997		

LOD limits of detection, Protocatechuic acid (PCA), Vanillic acid (VA), Gallic acid (GA), Propyl gallate (PG)