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Acute supplementation with blackcurrant extracts modulates cognitive functioning and inhibits monoamine oxidase-B in healthy young adults

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ABSTRACT

The consumption of berry fruits engenders a number of benefits in animal models, including improvements in cognitive performance, slowing of cognitive decline during natural ageing, and neuroprotection. These findings, along with limited human epidemiological evidence, suggest a potential role for the consumption of berry fruit polyphenols in improving human cognitive performance. The current study assessed the effects of two blackcurrant extracts on cognitive outcomes, mood, autonomic measures, peripheral and central monoamine tone, and anthocyanin bioavailability to plasma. A randomised, double-blind, placebo-controlled, crossover study was conducted using 36 healthy young participants (18–35 years). Findings from the intervention illustrate a cognitive benefit of acute blackcurrant supplementation in healthy young humans and the first description of a clinically significant inhibition of monoamine oxidase-B and monoamine oxidase-A using a commonly consumed fruit. These data also illustrate that compounds other than anthocyanins may be responsible for the observed *in vivo* MAO inhibition and that the degree of processing and the cultivar of blackcurrant fruit used substantially alter the neuroendocrinological and cognitive benefits conveyed.

1. Introduction

Epidemiological evidence suggests a relationship between flavonoid intake and cognitive decline/dementia (Letenneur, Proust-Lima, Le Gouge, Dartigues, & Barberger-Gateau, 2007; Nurk et al., 2009), with a specific benefit indicated for berries (strawberry and blueberry) that is not observed with other individual foods (Devore, Kang, Breteler, & Grodstein, 2012). Experimental support for this comes from literature

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demonstrating a slowing or reversal of natural cognitive decline in berry-fed rats (Casadesus et al., 2004; Joseph et al., 1999; Malin et al., 2011). Several different mechanisms of action have been proposed and investigated in an attempt to explain the modulation of memory in animal models, including anti-inflammatory and antioxidant responses and improvements to neural signalling (see Spencer, 2009 for review). Of particular relevance to the current study, anthocyanins, their aglycones and phenolic acids have been shown to have monoamine oxidase (MAO) inhibitory effects *in vitro* (Dreiseitel et al., 2009). The MAO enzymes directly produce hydrogen peroxide as they metabolise monoamines; inhibition of these may therefore reduce oxidative stress associated with this process and lead to increased concentrations of these monoamine neurotransmitters, essential for normal cognitive function and mood. Two subtypes of MAO exist: MAO-A and MAO-B. Central nervous system (CNS)-active MAO-B inhibitors are used in the treatment of neurodegenerative symptoms associated with Parkinson's disease and CNS-active MAO-A inhibitors are commonly used in the treatment of a variety of mood disorders, including depression. If inhibition of either MAO can be demonstrated within the CNS *in vivo* after the consumption of a MAO-inhibiting berry extract, mood and cognitive enhancing activities may also be attainable (Casacchia et al., 1984; Lonnqvist et al., 1994).

Only three published peer-reviewed intervention studies have demonstrated positive effects of berry consumption on human behaviour, affecting verbal memory and spatial memory after supplementation with Concord grape juice (Krikorian et al., 2012; Krikorian, Nash, Shidler, Shukitt-Hale, & Joseph, 2010) and blueberry juice (Krikorian, Shidler et al., 2010) in adults with age-related memory decline. There is, however, no published evidence pertaining to improvement of cognitive performance in healthy young adults.

One naturally rich source of anthocyanins that has received little attention in the cognition-related literature is blackcurrant (*Ribes nigrum*). Intact glucosides, galactosides and arabinosides of the berry anthocyanins and their associated metabolites have been found at extremely low concentrations, ranging from 0.2 to 1.5 ng/L, in the blood and urine of humans after oral ingestion of flavonoid rich berries such as blackcurrants, blueberries and boysenberries (Kay, Mazza, Holub, & Wang, 2004; Matsumoto, Ito, Yonekura, & Ichihashi, 2005; Mazza, Kay, Cottrell, & Holub, 2002). As well as anthocyanins, blackcurrant also contains an abundance of other phenolic structures in smaller quantities, which are able to exert physiological changes, such as the rate and pattern of glucose uptake from the small intestine (Bassoli et al., 2008; Cropley et al., 2012; Manzano & Williamson, 2010) and improved vascular function (Matsumoto, Takenami et al., 2005), which may have the potential to modulate human behaviour.

The aim of the present study was to explore the effects of an acute dietary supplementation with two blackcurrant extracts, with matched quantities of polyphenols and sugars but differing phenolic profiles, on attention, subjective mood, peripheral monoamines, prolactin and blood glucose. Anthocyanin bioavailability to plasma was also assessed at 2.5 h post-supplementation.

2. Materials and methods

2.1. Design

The study investigated the effects of two blackcurrant drinks, with balanced polyphenol content, on human cognitive function, mood and defined biochemical parameters. The study followed a double-blind, counterbalanced, placebo controlled, repeated measures design. Participants were randomly allocated to treatment orders as selected through a Williams Latin Square.

2.2. Participants

Thirty-six participants were recruited from Auckland, New Zealand, using opportunity sampling and received $NZ120 to recompense them for any expense they may have incurred to participate in the trial. Before participants were enrolled in the study, they attended a 90-min screening and training session. During this session, participants gave their written informed consent to participate in the study and were screened for any contraindications to the study. Demographic data can be found in Table 1. In brief, all participants reported themselves to be healthy, not pregnant, non-tobacco users. Participants were not using dietary supplements or prescribed, over-the-counter or recreational drugs (excluding the contraceptive pill), did not have any sensitivities to any of the study treatments, and had a body mass index below 35 kg/m^2. Participants also completed three repetitions of the study day tasks to ensure they met the required minimum standards (internally set) to participate in the study and to minimise practice effects. Sample size was determined using G Power statistical software (version 3.0). Thirty-six participants were needed to detect a true difference between treatment groups with a power of 90%. Recruitment for the trial ceased when 36 full sets of data were completed.

2.3. Treatments

Participants received three treatment drinks in an order dictated by random allocation to a counterbalancing (Williams Latin Square) schedule with at least one week of washout between visits. Extracts were assessed for the phytochemical constituents using the method described by Schrage et al. (2010). Intervention drinks contained either 0 mg of polyphenols (control) or 525 ± 5 mg of polyphenols per 60 kg of bodyweight from an anthocyanin-enriched blackcurrant extract (1.66 g of Just the Berries, Palmerston North, New Zealand (DelCyan™)) or from 142 mL of a cold-pressed blackcurrant fruit juice ('Blackadder' cultivar, cultivated and processed by Plant & Food

Table 1 – Mean participant characteristics.

Measure	Average measurement	SD	Range
Age (years)	24.8	3.93	18–34
Height (m)	1.72	0.12	1.52–1.97
Mass (kg)	70.93	17.82	44–116
BMI (m^2)	23.67	3.96	17–34

Table 2 – Phytochemical constituents of 'Blackadder' blackcurrant juice (mg/100 mL of raw juice and mg supplemented per 60 kg of body weight) and DelCyan extract (mg/g of raw powder and mg supplemented per 60 kg of body weight).

Compound	'Blackadder' juice mg/ 100 mL	DelCyan extract mg/g	'Blackadder' juice mg/ 60 kg	DelCyan extract mg/60 kg
Caffeoyl quinate	6.3	0.1	9	0.1
Caffeic acid glucoside	1.9	0.2	2.4	0.0
p-Coumaroyl quinate	3.6	0.4	5.4	0.7
Epigallocatechin	8.6	0	12	0
Delphinidin glucoside	24.1	44.6	34.2	73.8
Delphinidin rutinoside	115.9	107.4	164.4	178.2
Cyanidin glucoside	13.6	28.8	19.2	47.4
Cyanidin rutinoside	150.9	149	214.2	247.2
Myricetin rutinoside	15.6	4.5	22.2	7.2
Myricetin glucoside	2.1	0	3	0
Quercetin rutinoside	3.4	1.2	4.8	1.8
Quercetin glucoside	1.9	2.3	2.4	3.6
Quercetin pentoside	1.4	5.5	1.8	9
Myricetin	0.2	0.6	0.1	0.6
Vitamin C	168	0	100.8	0

Research, Auckland, New Zealand ('Blackadder' juice)). One hundred and forty-two millilitres of juice were yielded from approximately 150 g of fresh fruit, an amount that could realistically be consumed in one serving. The phytochemical content of each treatment is presented in Table 2 and the dose range of anthocyanins and other polyphenols used in the trial can be found in Table 3. The 'Blackadder' juice was frozen in 50-mL aliquots at –20 °C until the day of use. Anthocyanin stability of the 'Blackadder' juice extract at –20 °C was confirmed via high performance liquid chromatography (HPLC). Over the eight-week period, no significant loss of anthocyanins because of storage was observed. To ensure that all treatment drinks were sugar matched, the naturally occurring sugars glucose, fructose and sucrose in the 'Blackadder' juice were quantified via HPLC and the same concentrations were supplemented to the control and DelCyan treatments. The total volume of the drink was then made up to 200 mL (for a 60 kg person) with cold drinking water. All drink quantities were calculated per kg of body weight, resulting in differing volumes per person. In each case, all drinks contained 0.78 g of glucose (Healtheries), 0.13 g of fructose (Fruct-5, Absolute Ingredients Ltd), 0.09 g of a sucralose based sweetener (Splenda® granulated sweetener, Johnson & Johnson Ltd, New Brunswick, NJ, USA.) and 3.34-μL blackcurrant flavouring (NI #12220, Formula Foods Corporation Ltd. Christchurch, New Zealand) per kilogram of bodyweight. Drinks were coded and prepared freshly from frozen each morning by a third party who had no further part in the running of the study. No member of the investigation team was aware of the coding of the drinks until a blind-data review was completed.

2.4. *Cognitive and mood measures*

All cognitive measures and mood scales were delivered using the Computerised Mental Performance Assessment System (COMPASS, Northumbria University, Newcastle Upon Tyne, UK), a purpose-designed software application for the flexible delivery of randomly generated parallel versions of standard and novel cognitive assessment tasks, which has previously been shown to be sensitive to a range of nutritional interventions (Dodd, Kennedy, Riby, & Haskell-Ramsay, 2015; Kean et al., 2013; Stonehouse et al., 2013). For the purpose of behavioural analysis, three tasks were selected with the intention that attention performance and cognitive flexibility could be assessed. Seven repetitions of the digit vigilance task, Stroop task and rapid visual information task were completed in a fashion similar to that of the cognitive demand battery (Kennedy & Scholey, 2004), where subsequent repetitions of a 10-min battery are shown to induce mental fatigue incrementally. Mood scales were used at baseline, between each post-dose repetition of digit vigilance, Stroop and rapid visual information tasks and at the end of the cognitive tasks. The logical reasoning task was used at baseline and after the attentionally demanding

Table 3 – Anthocyanins and other phenolic compounds in each of the treatment conditions (mg per kg of body weight, average dose used in the intervention and dose range).

Treatment	Anthocyanins (mg/kg)	Anthocyanin average dose (mg)	Dose range	Other polyphenols (mg/kg)	Other polyphenols average dose (mg)	Dose range	total polyphenols (mg/kg)	Total polyphenols average dose	Dose range
Placebo	0	0		0	0		0	0	
'Blackadder'	7.786333	552.3336	344–906	0.66	46.93	29–77	8.44	599.3	373–983
DelCyan	8.05	571.03	356–937	0.28	19.86	12–32	8.32	590	368–968

cognitive battery to assess executive functioning (task order is illustrated in Fig. 1).

2.5. Study tasks

2.5.1. Digit vigilance

The digit vigilance task is a measure of sustained attention involving accurate selection of target stimuli. It focuses on alertness and vigilance while placing minimal demands on two other components of attention: selectivity and capacity. A single target digit was randomly selected and constantly displayed to the right of the screen. A series of single digits was presented in the centre of the screen at the rate of 80 per min. The participant was required to press the response key on the computer keyboard as quickly as possible every time the digit in the series matched the target digit. The task lasted 2 min and there were 30 stimulus–target matches. Task outcomes were accuracy (%), reaction time for correct responses (msec), and number of incorrect responses (false alarms).

2.5.2. Stroop

The Stroop test is a measure of attention, inhibition and cognitive flexibility. Participants were presented with a colour name. The colour name was written in a coloured ink, which could be the same as the colour name or different. Using a peripheral mouse, participants had to match the colour of the ink with the corresponding colour response button on the screen. Participants were presented with 60 stimuli. Task measures were accuracy (percentage correct) and reaction time (ms).

2.5.3. Rapid visual information processing (RVIP)

The RVIP task is a measure of sustained attention and working memory. The participants were required to monitor a continuous series of single digits for targets of three consecutive odd or three consecutive even single digits. The single digits were presented at the rate of 100 per min and the participants responded to the detection of a target string by pressing the response key on the computer keyboard as quickly as possible. The task was continuous and lasted for 5 min, with eight correct target strings being presented in each minute. The task was scored for percentage of target strings correctly detected, average reaction time for correct detections (ms), and number of incorrect responses (false alarms).

2.5.4. Logical reasoning

The logical reasoning test requires the participant to think logically and analytically and is a measure of cognitive flexibility. A series of statements referring to the relationships between two letters appeared on the screen one at a time (e.g. “a precedes b: ba”). Participants were required to decide if each statement correctly described the order of the two letters that followed it by pressing the designated response keys on the computer keyboard. There were 24 stimuli. Mean reaction times were measured in milliseconds, and accuracy of responses were recorded as percentages.

2.6. Mood

2.6.1. Bond–Lader visual analogue scales

Bond–Lader visual analogue mood scales (Bond & Lader, 1974), which have been utilised in a number of nutritional intervention studies, were employed (Camfield et al., 2013; Haskell, Kennedy, Milne, Wesnes, & Scholey, 2008). The reliability and validity of these visual analogue scales has been demonstrated (Ahearn, 1997). The scales comprise a total of sixteen 100-mm lines anchored at either end by antonyms (e.g. alert–drowsy, calm–excited) on which participants mark their current subjective position. Scores from the 16 Bond–Lader visual analogue scales were combined as recommended by the authors to form three mood factors: ‘alert’, ‘calm’ and ‘content’ (Bond & Lader, 1974).

2.6.2. Visual analogue scales

Following each repetition of the attentional demand battery, participants were asked to rate subjectively how mentally fatigued they felt and how difficult they found the cognitive tasks. The electronic visual analogue scales were anchored “not at all” on the left hand side of the scale and “extremely” on the right, with higher scores representing more mental fatigue/higher difficulty.

2.7. Study procedure

Each participant was required to attend a total of three study days, which were conducted at least seven days apart to ensure a sufficient wash out between conditions. During the week before, and throughout their participation in the study,

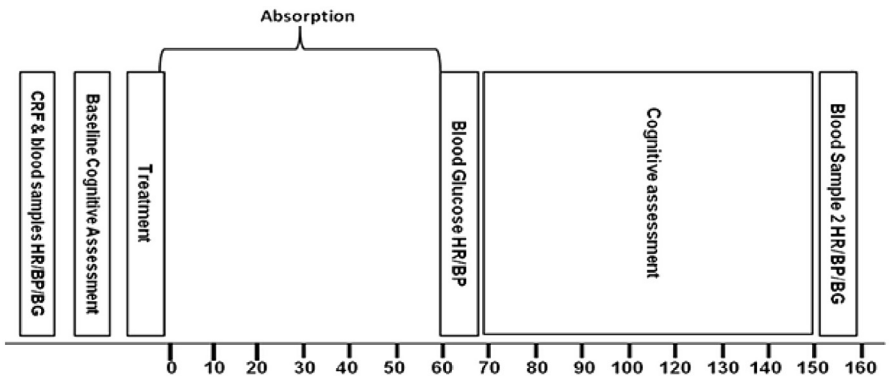

Fig. 1 – Study day running order. Scale depicts minutes post supplementation. CRF = case report form, HR = heart rate, BP = blood pressure, BG = blood glucose.

participants were asked to abstain from berry fruit consumption. Cognitive testing took place in a laboratory with participants visually and auditorily isolated from each other. On arrival at their first session, participants were randomly allocated to a treatment regime using a Latin square design that counterbalanced the order of treatments across the three active days of the study. On all three study days, participants arrived at the laboratory in the morning (0830 h), after an overnight fast (12 h), and firstly gave a 10-mL venous blood sample. Participants were then moved to the testing area where, after a 5-min seated resting period, heart rate, blood pressure (Omron M2 blood pressure monitor, Omron, Japan) and blood glucose were then measured. Participants then completed one repetition of the 10-min baseline cognitive assessment comprising of the digit vigilance task, the Stroop task, the RVIP task, mood scales and the logical reasoning task. This constituted the baseline measure for that day. Participants were then supplemented with one of the study treatments in the form of a drink, which they were given five minutes to consume. Drinks were served chilled and in a dark brown 300-mL plastic bottle with a straw to minimise the possibility of the participant recognising subtle differences in taste, look and mouth-feel between the treatments. After a 60-min resting absorption period, in which participants read in a waiting area, participants' blood pressure and heart rate were measured again and a second blood glucose reading was taken by finger prick. Participants then completed the post-dose cognitive assessment 65 min post consumption of the study treatments, a time when anthocyanins are known to be detectable in plasma after blackcurrant consumption (Matsumoto, Takenami et al., 2005). This paradigm consisted of seven repetitions of the attention tasks (digit vigilance, Stroop, RVIP) and mood scales. This lasted for 70 min and was followed by a single repetition of the logical reasoning central executive task. Participants then gave a third blood pressure reading and a third blood glucose reading before providing a second and final venous blood sample 130 min post dose. A diagram of the study visit running order is presented in Fig. 1.

The study received ethical approval from the New Zealand Regional Northern X Ethics board (application number NTX/10/07/066) and was conducted according to the Declaration of Helsinki (Goodyear, Krleža-Jeriç, & Lemmens, 2007).

2.8. Biochemical analysis

Venous blood samples (2 × 5 mL) were collected at baseline and 150 min after supplementation with treatments, which coincided with the end of the tasks. Samples were collected in 5-mL BD vacutainers© (Becton, Dickinson and Company, Plymouth, New Zealand). Both receptacles were treated with anticoagulants, one with lithium heparin (LH) and one with ethylenediaminetetraacetic acid (EDTA).

Whole blood samples treated with LH were immediately centrifuged (4 °C, 2400 × *g*, 10 min) (Hitachi Himac preparative ultracentrifuge model CP100MX, Hitachi Koki Co. Ltd. Hitachinaka City, Japan). Plasma was then extracted and aliquoted into 1-mL Eppendorf© tubes. Aliquots were spiked with 200 μL of 5% trifluoroacetic acid for the purpose of measuring plasma anthocyanin content. Plasma samples were stored at –80 °C until analysis was performed.

Whole blood samples treated with EDTA were used to isolate blood platelets using a method adapted from the one reported by Snell, Glanz, and Tabakoff (2002). Briefly, 3.5 mL of whole blood was added to 2 mL of phosphate buffered saline (PH 7.2) (PBS) containing 2 g of glucose per litre of solution (PBS solution) and gently inverted. The solution was then centrifuged at (22 °C, 600 × *g*, 3 min) and the supernatant placed on ice. The volume of the residual red cell pellet was restored to 7 mL with PBS solution, gently inverted to mix and centrifuged again (22 °C, 600 × *g* at 22 °C, 3 min); the supernatant was removed and pooled with the first supernatant fraction. This procedure was performed five times. The pooled supernatant fractions were then centrifuged (4 °C, 2000 × *g*, 10 min) and decanted leaving the platelet-enriched pellet which was then stored at –80 °C until the MAO-B activity assay was performed.

2.8.1. Monoamine oxidase-B (MAO-B) activity assay

A subset of eight participants provided sufficient blood samples for all the study time points to allow for MAO-B analysis. All time points needed to be available to allow for analysis using a within-subjects repeated measures design.

The isolated platelet-enriched pellet was slowly thawed on ice, re-suspended in 1 mL of PBS solution, sonicated with a probe sonicator (Microson ultrasonic cell disruptor, model XL2005, Qsonica LLC, Newtown, CT, USA.) for 15 s on ice and centrifuged (4 °C, 36,000 × *g*, 10 min). Sonication and centrifugation were then repeated, after which the supernatant was removed and the pellet consisting of lysed platelet membranes was re-suspended in sodium phosphate buffer supplied in the Amplex® Red Monoamine Oxidase-B Assay Kit (A12214 Invitrogen) (Life Technologies Ltd, Paisley, UK). The protein concentration of the lysed platelet solution was determined against a bicinchoninic acid standard curve by using the Pierce BCA protein assay (Thermo Fisher Scientific, New Zealand Ltd, North Shore City, Auckland, New Zealand) as per the manufacturer's instructions. Each sample was measured in triplicate and the average protein concentration was used. The lysed platelet membrane solution was re-suspended in sodium phosphate buffer to a final concentration of 150 μg/mL of protein.

Determination of MAO-B activity was conducted using the Amplex Red Monoamine Oxidase-B Assay Kit (A12214 Invitrogen), as per the manufacturer's instructions. One hundred microlitres of the diluted lysed platelet solution was added to a 96-well plate in triplicate. Two microlitres of the MAO-A inhibitor clorgyline were then added to each well that contained platelet membranes and incubated for 30 min at room temperature. During the incubation, 100 μL of H_2O_2 standards and the negative control (sodium phosphate buffer) were then added to each micro-plate in triplicate so a standard curve could be established. After the 30-min incubation, 100 μL of the Amplex Red working solution, comprising 5 mL of 5X reaction buffer stock solution and 20 mL of deionised water, were added to each well. The plate was then immediately placed into the microplate reader (FLUOstar Omega plate reader, BMG Labtech, Ortenberg, Germany) and set to incubate at 37 °C with an excitation wavelength of 530–560 nm and an emission wavelength of 590 nm. The micro-plate reader was programmed to take a reading every five minutes for one hour (13 readings in total). The 30-min reading was used to compare platelet MAO-B activity between the treatment groups.

2.8.2. Glucose

Blood glucose was measured with the use of an Accu-Check (Roche Healthcare Ltd., Auckland, New Zealand) blood glucose monitor via a finger prick blood sample at baseline, 60 min and 150 min post supplementation. All 35 participants who completed the study gave all required finger-prick blood samples.

2.9. Prolactin analysis

Prolactin was measured by diagnostic Medlab, Auckland, New Zealand. Prolactin was analysed in 300 μL of blood plasma collected in LH-treated vacutainers. Because of technical issues, only 20 sets of blood samples were available for prolactin analysis (eight control, seven DelCyan, five 'Blackadder' juice). For this reason, prolactin analysis was between subjects.

2.10. LC–MS analysis

The phytochemical composition of the blackcurrant extracts ('Blackadder' juice, DelCyan) and the control sample were determined by liquid chromatography–mass spectrometry (LC–MS) using a Shimadzu 2020 single-quadrupole mass spectrometer coupled to a Shimadzu 20-Series UFLC system with a UV-visible diode array detector (Auckland, New Zealand) using the method described by Schrage et al. (2010). The UV-visible chromatogram of DelCyan can be seen in Supplementary Fig. S1. MS data were used to aid identification of compounds. Concentrations of anthocyanins and monoamines in plasma at defined time points throughout the study were determined by LC–MS using a 5500 QTrap triple quadrupole/linear ion trap (QqLIT) mass spectrometer equipped with a TurboIon-Spray™ interface (AB Sciex, Concord, ON, Canada) coupled to an Ultimate 3000 UHPLC (Dionex, Sunnyvale, CA, USA).

2.10.1. LC–MS materials

Formic acid (Riedel-de Haën), ammonium formate and acetic anhydride (Fluka), and Hunig's base were purchased from Sigma Aldrich (Auckland, New Zealand). Optima LC/MS grade acetonitrile (Fisher Scientific) was purchased from Thermo Fisher (Auckland, New Zealand). Water was of Milli-Q grade. Analytical standards, dopamine, normetadrenaline, noradrenaline, adrenaline, 3,4-dihydroxyphenylglycol (DHPG), serotonin and homovanillic acid (HVA) were purchased from Sigma-Aldrich; phenylethylamine (PEA) from Acros Organics (Geel, Belgium); cyanidin 3-glucoside, cyanidin 3-rutinoside, delphinidin 3-glucoside and delphinidin 3-rutinoside from Polyphenols Laboratories (Sandnes, Norway); and malvidin 3-galactoside chloride from Extrasynthese (Genay Cedex, France). Deuterated acetic anhydride [d6] was purchased from Sigma-Aldrich and deuterated dopamine [d4] from CDN Isotopes (Quebec, Canada). Phree™ Phospholipid removal plates were purchased from Phenomenex (Torrance, CA, USA).

2.10.2. Anthocyanin analysis in plasma

A subset of 17 participants provided sufficient blood samples for all the study time points for anthocyanin analysis to be conducted.

Plasma samples (1 mL) were further acidified (1:4 6N HCl:5% formic $acid_{aq}$, 250 μL) and then spiked with malvidin galactoside (5 ng) as an internal standard. Samples were centrifuged (4 °C, 16100 RCF, 5 min) and proteins were removed by precipitation via addition of acetone (1:3) to an aqueous aliquot (600 μL). The samples were then chilled at –80 °C for 30 min prior to re-centrifuging (4 °C, 16,100 RCF, 5 min) and the acetone removed via evaporation. Further cleaning up to minimise the presence of phospholipids was achieved via liquid–liquid partition with hexane versus the aqueous sample. A final protein precipitation cleaning up of the aqueous aliquot (400 μL) with chloroform was performed prior to centrifuging (4 °C, 16100 RCF, 5 min). Two hundred microlitres of the aqueous phase were transferred to an autosampler vial for immediate analysis by LC–MS.

Anthocyanin separation was achieved on a Zorbax SB-C18 Rapid Resolution HD 2.1 × 100 mm ID 1.8 micron column (Agilent Technologies, Santa Clara, CA, USA) maintained at 70 °C. Solvents were (A) 5:3:92 (v/v/v) acetonitrile/formic acid/water and (B) 99.9:0.1 (v/v) acetonitrile/formic acid and the flow rate was 600 μL/min. The initial mobile phase, 100% A was held isocratically for 0.5 min, then ramped linearly to 10% B at 5 min, followed by another linear ramp to 90% B at 5.1 min and held for 1.9 min before resetting to the original conditions. Sample injection volume was 20 μL. MS data were acquired in the positive mode using a multiple reaction monitoring (MRM) method. The transitions monitored (Q1 and Q3), along with their optimised parameters (declustering potential (DP), entrance potential (EP), collision energy (CE) and collision cell exit potential (CXP)) are listed in Table 4.

Other operating parameters were as follows: dwell time, 100 ms; ionspray voltage, 2500 V; ion source temperature, 700 °C; ion source gas one, 60 psi; ion source gas two, 90 psi and curtain gas, 40 psi.

Quantification was performed using the internal standard ratio method using MultiQuant software v3.0 (AB Sciex). A total ion current plot showing multiple reaction monitoring transitions for the measured anthocyanins in plasma pre- and post-treatment can be found in Supplementary Fig. S2.

Table 4 – Multiple reaction monitoring transitions used for blackcurrant anthocyanins.

Q1	Q3	Name	DP	EP	CE	CXP
465	303	Delphinidin glucoside	70	10	30	10
611	303	Delphinidin rutinoside	130	10	50	30
449	287	Cyanidin glucoside	110	10	35	20
595	287	Cyanidin rutinoside	50	10	55	1
493	331	Malvidin galactoside (IS)	50	10	45	25

Declustering potential (DP), entrance potential (EP), collision energy (CE), collision cell exit potential (CXP).

2.10.3. *Monoamine analysis in plasma*

Eight monoamines were analysed by LC–MS from blood plasma following derivatisation. These were serotonin, dopamine, phenylethylamine, adrenaline, noradrenaline, normetadrenaline, 3,4-dihydroxyphenylglycol (DHPG) and homovanillic acid (HVA).

Plasma samples were treated to remove proteins and phospholipids and derivatised in two stages to acetylate alcohol and amine functional groups with acetic anhydride and alkylate-free carboxylic acids with Hunig's base prior to LC–MS analysis. A double derivatisation was found to be necessary to acetylate the less reactive alkyl hydroxyl groups. The schematic in Fig. 2 summarises the derivatisation of the different functional groups and the synthesis and use of labelled internal standards for each analyte to facilitate quantitation and to correct for matrix effects during analysis.

Briefly, each plasma sample (200 μL) was added to an individual well of a Phree Phospholipid removal plate already containing cold 600 μL acetonitrile, 100 μL acetic anhydride and 1 ng dopamine-d4 [internal standard (IS)]. The Phree plate was centrifuged at 500 × *g* for 30 min, a further 200 μL acetonitrile added to each well, and the plate centrifuged at 500 × *g* for a further 10 min. The filtrate was transferred to a 2-mL micro tube, 100 μL acetic anhydride added and heated at 50 °C. After 30 min, 20 μL Hunig's base were added to each sample, vortexed then heated for a further 60 min. Samples were then evaporated to near dryness with nitrogen at 40 °C. Samples were re-derivatised: 100 μL acetonitrile, 100 μL acetic anhydride and 10 μL Hunig's base heated for 40 min at 50 °C. Finally, to each sample 1 ng of the derivatised labelled internal standard monoamine mixture (d-IS) was added, and the sample made up to 1 mL with water and transferred to an autosampler vial ready for analysis.

Monoamine separation was achieved on an Atlantis® T3 150 × 2.1 mm^3 micron column (Waters Corp., Milford, MA, USA), maintained at 40 °C. Solvents were (A) MilliQ water + 0.03% ammonium formate + 0.1% formic acid and (B) acetonitrile + 0.1% formic acid and the flow rate was 0.6 mL/min. The initial mobile phase, 98% A, was held for 4 min then ramped linearly to 70% A at 11 min, 20% A at 14 min, and 0% A at 14.5 min and held for 5 min before resetting to the original conditions. Sample injection volume was 100 μL.

MS data were acquired in the positive mode using a scheduled MRM method. In some cases the ammonium adduct was the most abundant ion observed for Q1. The transitions monitored (Q1 and Q3), along with their optimised DP, EP, CE and CXP parameters are listed in Table 5.

Other operating parameters were as follows: ionspray voltage, 2500 V; ion source temperature, 700 °C; ion source gas one, 40 psi; ion source gas two, 50 psi and curtain gas, 50 psi.

Quantification was performed using the internal standard ratio method using MultiQuant software v3.0 (AB Sciex).

2.11. *Statistics*

Mood, cognitive scores and the physiological measures were analysed as 'change from baseline' using the SPSS 18 statistics package. Baseline differences were calculated for all measures using a one-way (treatment) ANOVA.

Two-way repeated measures ANOVAs (General linear model) (Treatment [control, DelCyan, juice] × completion [1 to 7] for attentional tasks and visual analogue scale outcomes OR Treatment [control, DelCyan, juice] × completion [1 to 2] for blood glucose and Bond–Lader) were conducted. Logical reasoning performance, platelet Monoamine Oxidase B activity, plasma monoamines and plasma anthocyanin concentrations were analysed by one-way (treatment) repeated measures ANOVA. Blood plasma prolactin was analysed using a one-way (treatment) between-subjects ANOVA. In all instances, Mauchly's test

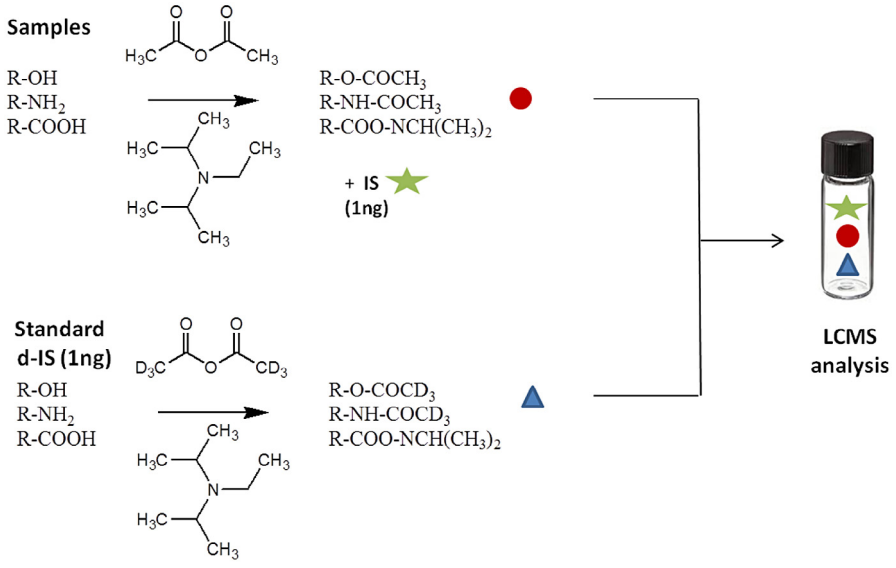

Fig. 2 – Schematic describing the derivatisation strategy and synthesis and use of internal standards for LC–MS analysis of monoamines in this study. IS, derivatised dopamine-d4 (1 ng) was added as internal standard to all samples at the beginning of extraction to correct for recovery. d-IS, a mixed monoamine standard derivatised with (d6) deuterated acetic anhydride (1 ng) was added to each sample prior to LC–MS analysis to facilitate quantitation and to correct for matrix effects during analysis.

Table 5 – Multiple reaction monitoring transitions used for monoamines and their isotopically labelled internal standard analogues.

Q1	Q3	Time	Name	DP	EP	CE	CXP
164	105	10.6	PEA	30	10	25	10
167	105	10.6	PEA [d3]	30	10	25	10
280	137	11.1	Dopamine	70	6.1	35	15
289	139	11.1	Dopamine [d9]	70	6.1	35	15
284	141	11.1	Dopamine [d4]	70	10	37	15
293	143	11.1	Dopamine [d4] [d9]	70	10	37	15
261	160	11.1	Serotonin	10	5	25	1
267	161	11.1	Serotonin [d6]	10	5	25	1
250	166	11.6	Normetadrenaline	50	9	25	15
256	168	11.5	Normetadrenaline [d9]	50	9	25	15
355	194	11.6	Noradrenaline	10	10	30	1
367	199	11.5	Noradrenaline [d12]	10	10	30	1
292	250	12.5	Adrenaline	170	10	20	1
301	257	12.5	Adrenaline [d12]	170	10	20	1
356.	237	13.4	DHPG	90	13	20	20
368	244	13.3	DHPG [d12]	90	13	20	20
308	224	13.9	HVA	110	10	25	20
311	225	13.9	HVA [d3]	110	10	25	20

Declustering potential (DP), entrance potential (EP), collision energy (CE), collision cell exit potential (CXP).

of sphericity was used to assess equality of the variances of the differences between factors. Where sphericity had been violated, Huynh–Feldt corrections for non-sphericity were implemented. Pairwise comparisons were conducted on all treatment-related effects with a p value <0.05 on the initial ANOVA to ascertain any differences between treatments for the whole session or, in the case of interactions, at each task repetition. Partial Bonferroni corrections were applied to protect for error against multiple comparisons; therefore, the p value was multiplied by the number of treatments being compared with the control. All *post hoc* p values are reported after corrections for multiple comparisons have been applied (×3 for anthocyanins and MAO-B and ×2 for all other comparisons).

3. Results

Prior to analysis of change from baseline data, mean pre-dose scores for all three treatments (control, DelCyan, Juice) for each outcome were subjected to a one-way repeated measures ANOVA. The only significant difference found was between active treatments for homovanillic acid. Data tables for all outcomes can be found in Supplementary Tables S1 and S2.

3.1. *Cognitive performance*

A one-way ANOVA was conducted on change from baseline data from the control condition for the outcome fatigue to ensure the sustained attention cognitive paradigm was indeed mentally fatiguing. A significant effect of repetition [$F(6,192) = 16.44$; $p < 0.0001$] confirmed that each subsequent repetition of the tasks caused an increase in rating of mental fatigue. All cognitive performance data and ANOVA outcomes can be found in Supplementary Table S1.

3.2. *Digit vigilance*

There was a significant treatment × repetition interaction on digit vigilance reaction time [$F(12,384) = 1.82$; $p = 0.044$] without any effect upon accuracy. Pairwise comparisons revealed an increase in speed of response after supplementation with the juice treatment at repetitions 1 ($p = 0.028$), 4 ($p = 0.011$) and 7 ($p = 0.038$) (Fig. 3a). There were no effects on any digit vigilance outcomes after supplementation with DelCyan.

3.3. *RVIP*

There was a significant main effect of treatment on RVIP accuracy [$F(2,62) = 5.87$; $p = 0.005$]. Pairwise comparisons showed an attenuation in the reduction of RVIP accuracy after supplementation with the DelCyan extract compared with the control ($p = 0.011$), irrespective of repetition. There were no significant effects on reaction time or false alarm (Fig. 3b). There were no effects on any RVIP outcomes after supplementation with juice.

3.4. *Bond–Lader*

There was a trend towards a treatment × repetition interaction on the Bond–Lader measure Alert [$F(2,64) = 2.61$; $p = 0.08$]. Pairwise comparisons revealed a higher alertness rating 150 min after supplementation with the DelCyan treatment ($p = 0.02$) compared with the placebo, with no effect on content or calm or content ratings (Fig. 4a). There were no effects of the juice treatment on any of the Bond–Lader outcomes.

3.5. *Mental fatigue*

There was a trend towards a treatment × repetition interaction on the visual analogue scale measure mental fatigue ($p = 0.08$). Pairwise comparisons showed an attenuation of the increase in feelings of fatigue at repetition 7 of the 70-min cognitive battery after supplementation with the DelCyan treatment ($p = 0.046$) (Fig. 4b).

3.6. *Blood glucose*

There was a significant main effect of treatment on blood glucose [$F(2,68) = 8.89$; $p < 0.001$]. Pairwise comparisons showed

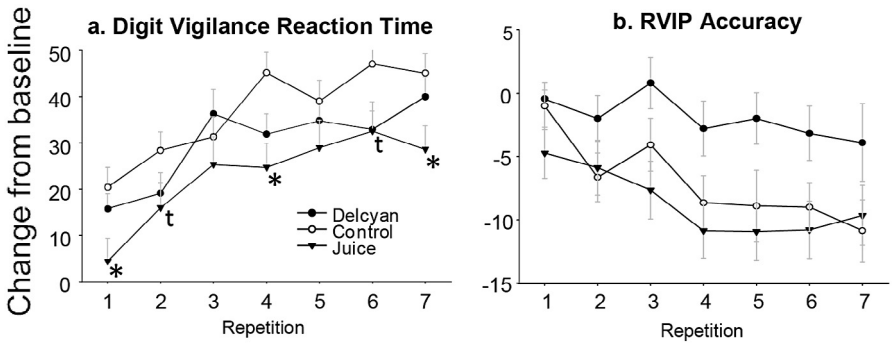

Fig. 3 – Mean change from baseline scores following control, DelCyan and juice treatments. A significant treatment by repetition interaction for digit vigilance reaction time (a) (*p < 0.05, t < 0.1) and a significant main effect of DelCyan on the rapid visual information processing task accuracy (b) are shown. Open circles depict control, closed circles depict DelCyan, and closed triangles depict the 'Blackadder' blackcurrant juice.

significantly higher blood glucose concentrations following supplementation with the juice treatment compared with the control ($p = 0.002$), irrespective of repetition (Fig. 5a). There were no significant effects following supplementation with DelCyan.

3.7. *Platelet MAO-B*

There was a significant effect of treatment on blood platelet MAO-B activity [$F(2,16) = 15.20$; $p < 0.001$]. Pairwise comparisons showed a decrease in platelet MAO-B activity after supplementation with the juice treatment compared with the control ($p < 0.001$) (Fig. 5b). There were no significant differences between active treatment groups. There was no effect of the DelCyan treatment on blood platelet MAO-B.

3.8. *Monoamines*

The repeated-measures ANOVA revealed a significant effect of treatment [$F(2,32) = 12.18$; $p < 0.001$] on plasma concentrations of normetadrenaline. Pairwise comparisons showed that concentrations of normetadrenaline were significantly higher after supplementation with the juice treatment than with the control ($p < 0.001$) and DelCyan ($p < 0.001$). There were no effects of DelCyan (Fig. 5c).

The repeated-measures ANOVA revealed a significant main effect of treatment [$F(2,32) = 21.30$; $p < 0.001$] on plasma concentrations of DHPG. Pairwise comparisons revealed that concentrations of dihydroxyphenylglycine (DHPG) were significantly higher after supplementation with the juice treatment than with the control ($p < 0.001$) and DelCyan ($p < 0.001$). There were no effects of DelCyan versus control (Fig. 5d).

Data for all physiological and biochemical outcomes can be found below in Table 6.

3.9. *Blood plasma anthocyanin concentrations*

The repeated-measures ANOVA revealed a significant effect of treatment on plasma concentrations of CY-GLU, CY-RUT, DEL-GLU and DEL-RUT [$F(2,34) = 27.5$; $p < 0.001$], [$F(2,34) = 33.7$; $p < 0.001$], [$F(2,34) = 112.51$; $p < 0.001$], [$F(1.45,25.25) = 96.26$; $p < 0.001$] respectively.

Plasma CY-GLU, CY-RUT, DEL-GLU and DEL-RUT concentrations were significantly higher after supplementation with the DelCyan treatment than with the control ($p < 0.001$, $p = 0.005$,

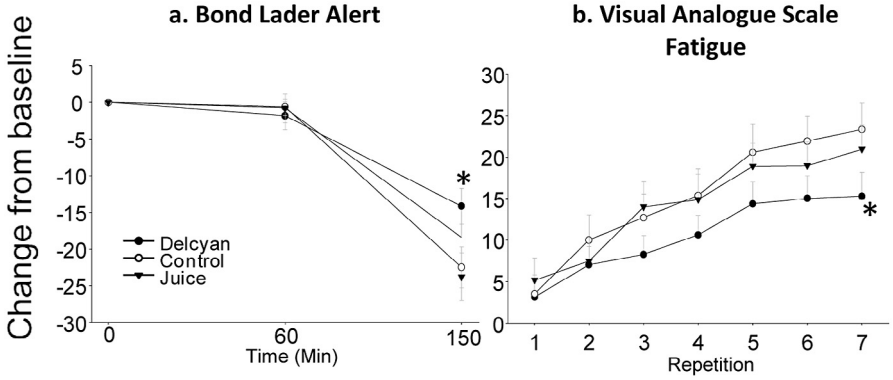

Fig. 4 – Mean change from baseline scores following control, DelCyan and juice treatments. A trend towards a significant treatment by repetition interaction for alert (a) and fatigue ratings (b) was evinced with ANOVA; significant effects of DelCyan compared with the control following pairwise comparisons are indicated (*p < 0.05). Open circles depict control, closed circles depict DelCyan™, and closed triangles depict the 'Blackadder' blackcurrant juice.

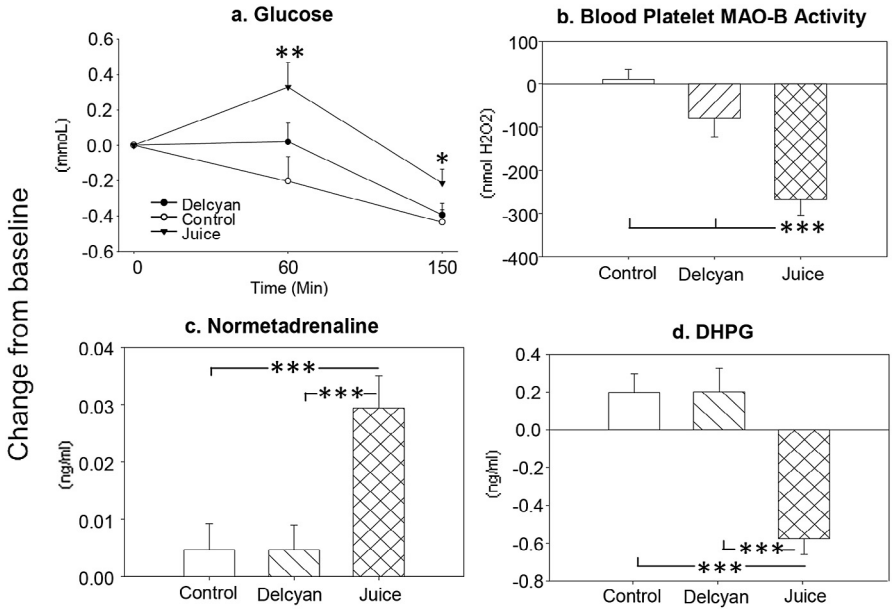

Fig. 5 – Mean change from baseline scores following control, DelCyan and juice treatments. Significant main effects of juice compared with the control are indicated (*p < 0.005). Open circles depict control, closed circles depict DelCyan and closed triangles depict the 'Blackadder' blackcurrant juice.**

$p < 0.001$, $p < 0.001$) or the 'Blackadder' juice treatment ($p < 0.001$, $p < 0.001$, $p < 0.003$, $p < 0.001$) respectively.

Plasma CY-GLU, CY-RUT, DEL-GLU and DEL-RUT concentrations were also significantly higher after supplementation with the 'Blackadder' juice treatment than with the control ($p < 0.001$), ($p < 0.001$), ($p = 0.003$) and ($p < 0.001$) respectively. A graphical representation of anthocyanin concentrations in blood plasma is presented in Fig. 6a.

Following supplementation, the repeated-measures ANOVA revealed a significant effect of the treatment on combined concentrations of CY-GLU, CY-RUT, DEL-GLU and DEL-RUT in blood plasma (combined anthocyanin concentration is the total amount of anthocyanins measured in blood plasma after supplementation with the study treatment) after consumption of the study treatments [$F(2,32) = 105.34$; $p < 0.0001$]. Pairwise comparisons revealed that combined anthocyanin concentrations were significantly higher after consumption of the DelCyan ($p < 0.001$) and juice ($p < 0.001$) treatments than with the control. Concentrations were also significantly higher after supplementation with the DelCyan extract than with the 'Blackadder' juice extract ($p < 0.001$). A graphical representation of combined anthocyanin concentrations in blood plasma is presented in Fig. 6b. A table containing all Means and SDs for the amount of measured anthocyanins given to the participants and the amount found in blood plasma can be found in Table 7.

4. Discussion

The current study has outlined evidence of distinct positive modulation of behaviour following administration of each of the two blackcurrant extracts compared with the control, with no negative effects caused by either active extract. Improvements in RVIP accuracy were found after supplementation with the DelCyan extract, and improvements in reaction time on the digit vigilance task were found after supplementation with the 'Blackadder' juice extract. The 'Blackadder' juice treatment further induced a number of robust neuroendocrinological and physiological effects not present following the DelCyan treatment. These comprised an almost complete inhibition of blood platelet MAO-B activity (96%), and a significant reduction in plasma normetadrenaline concentration (60%) and an increase in DHPG concentration (~35.5%) when measured 2.5 hours after supplementation. The 'Blackadder' blackcurrant juice treatment also induced a significant and sustained (over both time points) increase in blood glucose concentration compared with the control at 60 and 150 min, despite the treatments being sugar matched.

An increase in accuracy was shown during the RVIP task after supplementation with the DelCyan treatment, irrespective of task repetition, with no evidence of slowed reaction times. With regards to the juice treatment, there was evidence of an attenuation of the increase of digit vigilance reaction times seen with repeated testing, with no evidence of decreased accuracy. This improvement was seen during repetitions 1, 4 and 7 (70, 100 and 140 min post supplementation, respectively). Further evidence for a modulation of behaviour following the blackcurrant extracts comes from non-significant trends observed on the treatment × repetition ANOVAs for Bond–Lader alertness ratings and mental fatigue visual analogue scales after the consumption of the DelCyan supplement. These indicated a pattern of attenuation in decreased self-reported alertness and increased ratings of fatigue following supplementation with the DelCyan treatment but only reached statistical significance after the final repetition of the 70-min attentionally demanding cognitive battery.

Table 6 – Mean pre-dose baseline and change from baseline scores, standard deviations and ANOVA outcomes for each physiological parameter following supplementation with the control, DelCyan and 'Blackadder' blackcurrant juice drink.

Measure	N	Treatment	Baseline		Repetition 1		Repetition 2		Effect of Treatment	Treatment × repetition interaction
			Mean	SD	Mean	SD	Mean	SD		
Heart rate (BPM)	35	Control	69.6	11.76	–2.26	9.12	–7.46	10.12	F = 1.00 p > 0.1	F = 0.72 p > 0.1
		DelCyan	72.14	12.07	–4.2	7.26	–8.14	7.87		
		Juice	72.46	11.89	–4.09	9.81	–10.2	11.2		
Diastolic blood pressure (mmHg)	35	Control	76.83	7.64	–2.17	6.88	0.71	10.6	F = 0.04 p > 0.1	F = 1.64 p > 0.1
		DelCyan	76.89	8.31	–1.91	6.22	–0.11	6.76		
		Juice	74.63	7.32	0.54	6.13	0	8.32		
Systolic blood pressure (mmHg)	35	Control	121.3	12.62	–3.09	9.3	–0.29	10.7	F = 0.16 p > 0.1	F = 1.53 p > 0.1
		DelCyan	122.7	13.65	–2.2	9.77	–0.6	11.37		
		Juice	122.9	11.74	–1.86	11.77	–3.51	14.41		
Glucose (mmoL)	35	Control	4.71	1.22	–0.2	0.8	–0.43	0.42	F = 8.89 p < 0.001	F = 2.45 p=0.09
		DelCyan	4.68	1.13	0.02	0.62	–0.39	0.39		
		Juice	4.71	1.15	0.33	0.82	–0.21	0.47		
Prolactin (IU/L)	8	Control	271.6	70.52	–103.2	79.85			F = 1.6 p > 0.1	
	7	DelCyan	315.4	135.9	–121.3	116.36				
	5	Juice	353.4	105.3	–196.2	77.42				
MAO-B (nmol H_2O_2)	8	Control	258	112.3	11.32	68.28			F = 15.22 p < 0.001	
		DelCyan	220.6	131.2	–78.06	131.88				
		Juice	273.1	114.1	–267.4	109.82				
Total anthocyanins (nM/L)	17	Control	0	0	–0.02	0.35			F = 105.54 p < 0.001	
		DelCyan	0	0	22.03	7.62				
		Juice	0	0	15.16	4.6				
Phenylethylamine (ng/mL)	17	Control	0.05	0.18	0.01	0.04			F = 0.96 p > 0.1	
		DelCyan	0.04	0.14	0.02	0.08				
		Juice	0.09	0.35	0.01	0.02				
Dopamine (ng/mL)	17	Control	0.02	0.02	0	0.02			F = 0.39 p > 0.1	
		DelCyan	0.02	0.01	0	0.01				
		Juice	0.02	0.01	0	0.01				
Serotonin (ng/mL)	17	Control	1.9	1.34	–0.15	2.25			F = 0.75 p > 0.1	
		DelCyan	2.37	2.06	–0.73	1.26				
		Juice	2.23	2.3	–0.62	2.03				
Normetadrenaline (ng/mL)	17	Control	0.05	0.02	0	0.02			F = 12.19 p < 0.001	
		DelCyan	0.06	0.03	0	0.02				
		Juice	0.05	0.02	0.03	0.02				
Noradrenaline (ng/mL)	17	Control	0.3	0.1	0.15	0.16			F = 0.97 p < 0.1	
		DelCyan	0.35	0.1	0.11	0.15				
		Juice	0.33	0.11	0.09	0.17				
Adrenaline (ng/mL)	17	Control	0.02	0.02	0.01	0.03			F = 2.189 p > 0.1	
		DelCyan	0.02	0.03	0	0.01				
		Juice	0.02	0.02	–0.01	0.02				
DHPG (ng/mL)	17	Control	1.61	0.36	0.2	0.41			F = 21.30 p=0.001	
		DelCyan	1.6	0.34	0.2	0.51				
		Juice	1.63	0.37	–0.58	0.34				
Homovanillic acid (ng/mL)	17	Control	21.42	12.21	–4	14.9			F = 0.55 p > 0.1	
		DelCyan	27.45	16.7	–7.67	10.34				
		Juice	17.12	8.01	–5.31	7.63				

Highlighted in grey: the variable/analysis was not assessed at that repletion.

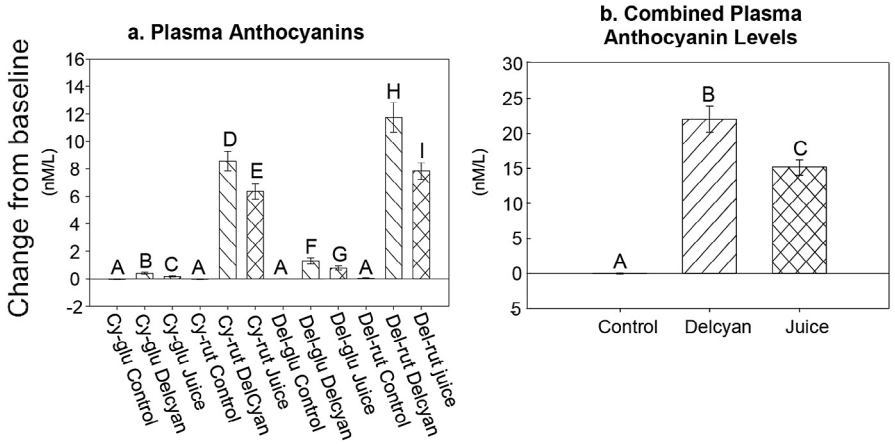

Fig. 6 – Grouped mean blood plasma anthocyanins for each anthocyanin (a) and combined plasma anthocyanins ((b) combined anthocyanin is the sum of measured anthocyanins for each condition). Means with a different letter are significantly different from each other (cy-glu = cyanidinglucoside, cy-rut = cyanidinrutinoside, del-glu = delphinidinglucoside, del-rut = delphinidinrutinoside).

There is evidence of reduced advanced glycation end products (Chen et al., 2014) and strong radical scavenging activity (Jia, Xiong, Kong, Liu, & Xia, 2012) *in vitro* by blackcurrant extracts, and direct cellular and molecular interactions of flavonoids on rodent brains (Spencer, 2009), as well as changes in central (Francis, Head, Morris, & Macdonald, 2006) and peripheral (Matsumoto, Takenami et al., 2005) vascular function in humans after consumption of flavonoid-rich fruits. However, definitive mechanisms driving the behavioural effects in the present study are currently unknown, especially after supplementation with the DelCyan treatment, which had no significant effect upon any of the neuroendocrinological outcomes measured.

In line with previous human data, plasma anthocyanin levels were significantly increased 2.5 hours after supplementation with both blackcurrant treatments compared to control (Matsumoto, Takenami et al., 2005; Mazza et al., 2002; Nielsen, Dragsted, Ravn-Haren, Freese, & Rasmussen, 2003). Despite being supplemented with ~45 mg more cyanidin (CY-GLU + CY-RUT) than delphinidin (DEL-GLU + DEL-RUT), plasma levels of delphinidin were significantly greater than cyanidin. These findings are similar to those of Matsumoto et al. (2001) and Matsumoto, Takenami et al. (2005) and provide further evidence towards a greater level of absorption of DEL-GLU and DEL-RUT than CY-GLU and CY-RUT when administered orally to human participants.

Measured blood plasma anthocyanins were also greater after supplementation with the DelCyan treatment compared with the juice treatment, as expected. When results from all four measured anthocyanins were combined, there was a 30% increase in plasma concentration following the DelCyan treatment compared with the juice treatment. However, this extract contained 20% more of the measured anthocyanins than the juice drink. The significant difference in blood plasma anthocyanin concentration resulting from the two blackcurrant treatments may relate to different metabolisms of the two

Table 7 – Means and SDs for the amount of measured anthocyanins given to the participants and the amount found in blood plasma (change from baseline) 150 min post supplementation with the DelCyan and 'Blackadder' blackcurrant juice treatments.

Treatment	Anthocyanin	Amount supplemented mg/kg body weight	Amount supplemented mg/60 kg of body weight	Average amount in plasma (nM)
DelCyan	Cyanidin glucoside	0.8	48	0.4 ± 0.3
	Delphinidin glucoside	1.2	73.8	1.3 ± 0.9
	Cyanidin rutinoside	4.1	247.2	8.6 ± 2.9
	Delphinidin rutinoside	2.9	178.2	11.7 ± 4.5
'Blackadder' juice	Cyanidin glucoside	0.3	19.2	0.2 ± 0.1
	Delphinidin glucoside	0.5	34.2	0.8 ± 0.6
	Cyanidin rutinoside	3.5	214.2	6.4 ± 2.3
	Delphinidin rutinoside	2.7	164.4	7.8 ± 2.5
Control	Cyanidin glucoside	0	0	0.0 ± 0.1
	Delphinidin glucoside	0	0	0.0 ± 0.01
	Cyanidin rutinoside	0	0	0.0 ± 0.1
	Delphinidin rutinoside	0	0	0.0 ± 0.1

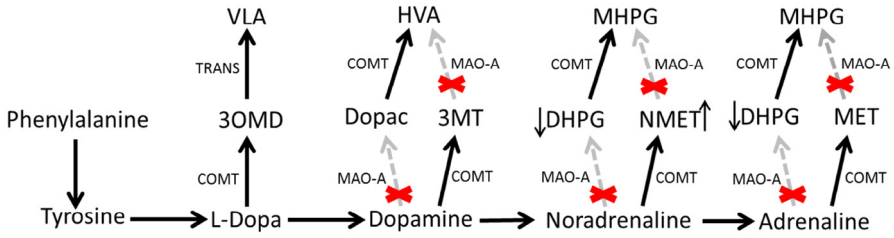

Fig. 7 – Potential inhibitory actions of the 'Blackadder' blackcurrant juice upon enzymes responsible for the metabolism of dopamine, noradrenaline and adrenaline. MAO = monoamine oxidase, HVA = homovanillic acid, MHPG = 3-Methoxy-4-hydroxyphenylglycol, DHPG = 3,5-dihydroxyphenylglycine, NMET = normetadrenaline, MET = metadrenaline, COMT = Catechol-O-methyl transferase. ✖ depicts a potential inhibition of the enzyme. ↑/↓ indicates a significant increase or decrease in the specific monoamine.

extracts, where the juice extract appears to be more bioavailable than the powdered and rehydrated extract. Conversely, the powdered extract may have been absorbed more quickly and its anthocyanins were already metabolised into aglycones at the time of blood sampling, which were not measured in our analysis. In line with the observations of Fernandes, Faria, Calhau, de Freitas, and Mateus (2014) these bioavailability data highlight the need for further research to assess the impact of food matrix and post-harvest preparation of foods on anthocyanin absorption. It must be noted that vitamins and minerals, other than L-ascorbic, were not quantified in any of the study treatments. However, to our knowledge there are no reported cognitive or behavioural effects of acute vitamin or mineral supplementation. Given that the major phenolic constituents of each treatment were anthocyanins, and that both extracts affected attention-based tasks, this may indicate that the effects of blackcurrant upon attention processing are directly related to their anthocyanin content, an acute effect which has previously been indicated in children aged 7–9 years (Whyte & Williams, 2012). The specific demands of the two attention tasks are, however, not equal, with a higher demand both in processing and in duration of the RVIP task than in the digit vigilance task. The RVIP contains a higher working memory element than the digit vigilance task, potentially indicating changes in working memory processing as well as attention, a cognitive outcome which has previously been shown to be sensitive to flavonoid-rich cocoa (Scholey et al., 2010) and *Ginkgo biloba* extract (Rigney, Kimber, & Hindmarch, 1999). However, until replication of the behavioural effects presented in the current study has been achieved, it is difficult to elaborate further at this point.

In terms of MAO-B activity, this is the first demonstration of a clinically significant inhibition of platelet MAO-B following blackcurrant supplementation. Central MAO-B inhibitors have been used for several decades for the treatment of depressive disorders and neurodegenerative diseases (Youdim & Bakhle, 2006) and have also been shown to improve cognitive processing when given to non-demented Parkinson patients (Hanagasi et al., 2011). MAO-B inhibitors also have the potential to attenuate the breakdown of endogenous neurotransmitters, reducing concentrations of H_2O_2 associated with the deamination of monoamines (Pizzinat, Copin, Vindis, Parini, & Cambon, 1999). Although the current study measured MAO-B inhibition only in peripheral tissue, if the inhibition could be shown to be centrally active, the clinical applications of a MAO inhibitor from a commonly consumed fruit could be enormous. Potential applications include attenuating cognitive decline associated with natural ageing, as well as in clinical populations, including those suffering from early-stage Parkinson's disease, who are known to respond favourably to MAO inhibitors (Hanagasi et al., 2011). DHPG, a metabolite largely determined by the MAO-A-dependent metabolism of noradrenaline in the periphery (Scheinin, Karhuvaara, Ojala-Karlsson, Kallio, & Koulu, 1991), which is a marker for reduced MAO-A activity after administration of pharmacological MAO-A inhibitors (Zimmer, 1990), was also found to be reduced after consumption of the 'Blackadder' blackcurrant juice extract in the current study. This effect was not seen after consumption of the DelCyan extract, highlighting that, in addition to MAO-B inhibition, the 'Blackadder' juice treatment possesses peripheral MAO-A-inhibitory properties. Interestingly, as blood plasma anthocyanin concentrations were higher after consumption of the DelCyan extract, these results are not consistent with published *in vitro* data, where anthocyanins have been shown to be inhibitors of both MAO subtypes (Dreiseitel et al., 2009), potentially indicating that another constituent of the 'Blackadder' extract, which is not present in the DelCyan extract, was inhibiting MAO in the present study, or that the effect was of differential anthocyanin absorption and metabolism rates between the two treatments, as discussed above.

These changes in plasma DHPG did not coincide with an accumulation of plasma adrenaline or plasma noradrenaline concentrations in the current study, which is in line with results of previous research investigating acute supplementation of MAO inhibitors in humans (Illi, Sundberg, Ojala-Karlsson, Scheinin, & Gordin, 1996). In addition to decreased concentrations of plasma DHPG, indicating peripheral MAO-A inhibition, we also observed an increase in plasma normetadrenaline, a metabolite of noradrenaline via catechol-O-methyl transferase (COMT). This increase is potentially indicative of increased noradrenaline breakdown through COMT as a result of inhibition of the peripheral MAO-A enzyme. A diagram depicting potential inhibition pathways is presented in Fig. 7. Also related to this MAO-inhibitory effect is a non-significant modulation of plasma prolactin observed in the current study, where post-dose prolactin was lower after consumption of the 'Blackadder' juice extract

than in the control. Although gamma-aminobutyric acid (GABA), serotonin, adrenaline and noradrenaline are slight rate-limiting factors of prolactin secretion, dopamine is the most important hypothalamic prolactin-inhibiting factor (Fitzgerald & Dinan, 2008), indicating that general and potentially central dopaminergic tone could have been affected by supplementation with the 'Blackadder' juice drink. Although these prolactin findings are hindered by a small sample size and between-subjects design, they illuminate the need for further research.

The blackcurrant juice treatment also showed a significant (over both time points) increase in blood glucose compared with the control, despite being sugar matched, an effect not seen with supplementation by the DelCyan treatment. Blood glucose was elevated by 0.53 mmol/L at 60 min and 0.23 mmol/L at 150 min post supplementation of the juice treatment. Although these results must be interpreted with caution as there were only two post-dose blood glucose measurements, this result shows a clear effect of the juice treatment on blood glucose concentrations. Based upon the current findings, the effect on glucose appears to resemble the pattern found after supplementation with berry puree, where the peak in blood glucose concentrations following a glucose load when combined with a berry puree was reduced, resulting in a higher blood glucose reading at 1 h after supplementation (Torronen et al., 2010). Another difference between the study treatments was the presence of the phenolic acids caffeoyl quinate, caffeic acid glucoside and p-Coumaroyl quinate at 1.1 mg in the DelCyan treatment and 16.5 mg in the juice treatment per 60 kg of bodyweight, which could provide a further slowing of glucose transport from the gut via direct inhibition of intestinal epithelial glucose transporters, described by Manzano and Williamson (2010). A more thorough investigation needs to be completed to ascertain a full post-supplementation blood glucose profile.

The findings of the present study demonstrate, for the first time, a positive modulation of behaviour in a young and healthy adult cohort after supplementation with two different blackcurrant extracts. This is also the first evidence of a clinically significant reduction in MAO activity following ingestion of a commonly consumed fruit. The results suggest that the MAO inhibition found in this study cannot be wholly responsible for the behavioural effects observed, as both active conditions positively influenced attention-based cognitive tasks, whereas only the juice treatment inhibited MAO-A and MAO-B. The finding of more robust effects on attention following DelCyan supplementation, containing higher concentrations of anthocyanins, may indicate that these effects are attributable to the anthocyanin content and that any effects on MAO are independent of these. The possibility that a MAO-A- and MAO-B-inhibiting blackcurrant drink will exert favourable effects on cognitive modulation of clinical and non-clinical populations deserves further investigation. More exploration therefore needs to be undertaken to ascertain if other cognitive paradigms, especially those that have previously been shown to be sensitive to flavonoid-rich nutritional interventions in rats and humans, specifically memory tasks and paradigms sensitive to changes in concentrations of dopamine, are modulated after supplementation with a MAO-inhibiting blackcurrant juice.

Conflicts of interest

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Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.jff.2015.06.005.

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