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Tart Montmorency cherries (*Prunus Cerasus L.*) modulate vascular function acutely, in the absence of improvement in cognitive performance

Keane, KM[1], Haskell-Ramsay, CF[2], Veasey, RC[2] and Howatson, G[1,3]

[1]*Department of Sport, Exercise and Rehabilitation, Faculty of Health and Life Sciences, Northumbria University, Newcastle Upon Tyne, UK*

[2]*Brain, Performance and Nutrition Research Centre, Faculty of Health and Life Sciences, Northumbria University, Newcastle upon Tyne NE18ST*

[3]*Water Research Group, School of Environmental Sciences and Development, Northwest University, Potchefstroom, South Africa*

Corresponding Author:

Karen Keane

Department of Sport, Exercise and Rehabilitation

Faculty of Health and Life Sciences

Northumbria University

Newcastle upon Tyne, UK

Tel: 00 44 (0) 191 227 7086

Email: k.keane@northumbria.ac.uk

Tart cherries, vascular and cognitive function

Keywords: Tart cherries, cerebral blood flow, blood pressure, cognitive performance

Abstract

Cerebral blood volume and metabolism of oxygen declines as part of human ageing and this has been previously shown to be related to cognitive decline. There is some evidence to suggest that polyphenol-rich foods can play an important role in delaying the onset or halting the progression of age-related health disorders such as cardiovascular and Alzheimer's disease, and to improve cognitive function. In the present study, an acute, placebo-controlled, double blinded, cross-over, randomised Latin square design study with a wash-out period of at least 14 days was conducted in twenty-seven middle aged (defined as 45-60 years) volunteers. Participants received either a 60 mL dose of a Montmorency tart cherry concentrate (MC), which contains 68.0 ±0.26 mg cyanidin-3-glucoside /L, 160.75 ± 0.55 mean gallic acid equiv/L and 0.59 ± 0.02 mean Trolox equiv/L, respectively or a placebo (PLA). Cerebrovascular responses, cognitive performance and blood pressure were assessed at baseline and 1, 2, 3 and 5 h following consumption. There were significant differences in concentrations of total and oxy-haemoglobin during the task period 1 h post MC consumption ($p \leq 0.05$). Furthermore, MC consumption significantly lowered SBP ($p \leq 0.05$) over a period of 3 h, with peak reductions of 6 ± 2 mmHg at 1 h post MC consumption relative to the placebo. Cognitive function and mood were not affected. These results show that a single dose of MC concentrate can modulate certain variables of vascular function; however this does not translate to improvements in cognition or mood.

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Introduction

Montmorency tart cherries (*L. Prunus Cerasus*) and their derivatives are a functional food that are high in numerous phytochemicals [1; 2; 3; 4; 5] that include the flavonoids isorhamnetin, kaempferol, quercetin, catechin, epicatechin, procyanidins, and anthocyanins [6; 7]. It has been previously shown that Montmorency tart cherries attenuate inflammation [1], oxidative stress [8; 9] and improve aspects of vascular function [3]. One property underlying the potential vascular effects of tart cherries is an ability to modulate blood flow parameters. Cherry extracts have been shown, in cell and animal models, to exert a range of cardio-protective effects that include increasing nitric oxide production and antioxidant status, reducing lipid oxidation and inhibiting inflammatory pathways [1; 4]. Even more recently, Keane et al. [3] demonstrated an increase in plasma phenolic acids (vanillic and protocatechuic) following Montmorency tart cherry consumption in humans; these compounds were also shown to modulate vascular smooth muscle cell behaviour *in vitro*. In a subsequent study, Keane and colleagues demonstrated that circulating phenolic metabolites derived from Montmorency tart cherry juice are, at least in part, responsible for an acute reduction in systolic blood pressure in men with early hypertension [10].

Aging is associated with deficits in motor function, which include decreases in balance, muscle strength, coordination, and cognitive function, especially in tasks that require the use of spatial learning and memory. This has been suggested to be caused by a concurrent decline in cerebral blood volume and metabolism of oxygen which also occurs as a result of aging [11]. These decrements have been reported in numerous studies in both animals [12; 13] and humans [14; 15]. A large number of dietary interventions using polyphenol-rich foods or beverages, in particular those using tea [16], Gingko Biloba [17], cocoa [18] and blueberry [19], have demonstrated beneficial effects on memory and learning in both animals and humans. Although it is not clear whether tart cherries can decrease the risk of neurodegenerative aging or diseases such as Parkinson's and Alzheimer in humans, studies with animal models are more positive and suggest that the phenolic compounds found in tart cherries, may exert their beneficial effects through their ability to lower oxidative stress and anti-inflammatory properties or by altering directly the signalling involved in neuronal communication, calcium buffering ability, stress signalling pathways among others [19; 20].

Seymour et al [21] showed that intake of 1% tart cherry diet significantly reduced stroke-related phenotypes in rats. Tart cherry intake also reduced brain NFκB activity and the related pro-inflammatory transcripts. Interestingly in 2015, Kirakosyan and colleagues [22] confirmed that tart cherry anthocyanins cross the blood-brain barrier. In a more recent addition to the literature, thirty 19-month-old male Fischer 344 rats who received either a control diet or a diet supplemented with 2%

Montmorency tart cherry for six weeks were examined. Results showed that although there were no changes on motor performance, tart cherry supplementation significantly improved working memory of aged rats [23]. However, there is a paucity of data from human trials to extrapolate these findings to hominids.

Caldwell et al., [24] previously demonstrated that regardless of dose, cherry juice had no acute impact on cognitive function in young people, older people or dementia patients. They concluded that although cherry juice may have an acute impact on cardiovascular function, there was no change in cognitive performance 6 h post consumption. Contrastingly, a chronic supplementation study [25] reported that the daily consumption of sweet cherries for 12 weeks improved cognitive performance across almost all tasks in older adults with mild-to-moderate dementia; this group showed improvements for category verbal fluency and tasks relating to verbal learning and memory and concluded the positive changes have clinical relevance for these cognitive improvements. It would therefore appear that the cerebrovascular response required to elicit measurable changes in cognitive function can only be achieved with longer term dosing strategies [26]. Contrary to this theory, two recent additions to the literature suggest that acute supplementation has the ability to improve aspects of cognitive function. Acute blackcurrant supplementation was shown to improve both digit vigilance and rapid visual information processing in healthy younger humans [27]. Similarly, acute wild blueberry supplementation was shown to improve final immediate recall, delayed word recognition, and accuracy on cognitively demanding incongruent trials in the interference task in in children [28]. Therefore, it is possible that Caldwell and colleagues reported no impact of cherry supplementation on cognitive function as they used sweet cherries as an intervention. It has previously been speculated that sweet cherries are not as rich in phytochemical compounds as tart cherries[6].

Polyphenol-rich foods have also been reported to improve cerebral haemodynamics assessed by near infrared spectroscopy (NIRS) and function magnetic resonance imaging (fMRI). Wightman and colleagues [29] assessed the effect of EGCG on cerebral blood flow using NIRS in healthy adults. Results suggested that 135 mg of EGCG caused a reduction in total haemoglobin, a proxy for cerebral blood flow during cognitive tasks relative to the placebo. Changes in cerebral blood flow has also been demonstrated following resveratrol [30] and beetroot supplementation [31]. Krikorian et al., [32] used fMRI to examine the effect of Concord grape juice on neurocognitive function. Sixteen adults aged >68 y with mild age-related memory decline were supplemented with either a grape juice (444 ml average) containing on average, 209mg of polyphenols, or a sugar matched placebo for 16 weeks. Results found that after 16 weeks, there were reductions in semantic interference on memory tasks and relatively greater activation in anterior and posterior regions of the right hemisphere in the grape juice treated group. Similarly, people with mild memory complaints, who drank pomegranate juice daily, performed better on memory task compared to a placebo and displayed an increase in brain activation measured by fMRI [33]. Very little has been reported on the effect of acute polyphenol

supplementation on cerebral haemodynamics, with the majority of this work carried out with flavanol-rich cocoa [34; 35]. At present, no attempt been made to examine the haemodynamic response to acute tart cherry supplementation.

Notwithstanding, given that Montmorency tart cherries are capable of modulating human vascular function (particularly in relation to blood pressure and vascular smooth muscle behaviour), we hypothesised that cerebral blood flow could also be acutely modulated and consequently improve cognitive performance in humans. Therefore, the aim of the present study was to assess the impact of Montmorency tart cherry juice consumption on pre-frontal cortical haemodynamics, cognitive function and blood pressure in middle aged adults.

Methods

Participants

Thirty healthy middle aged (defined as 45-60 years) adults (10 female, 20 male, 28 right-handed, 2 left-handed) were recruited to take part in the study; the mean ± SD age, stature, mass and BMI were 50 ± 6 years, 170.7 ± 9.1 cm, 76.0 ± 16.0 kg and 26.1 ± 4.9 kg/m^2, respectively. All participants were in apparent good health as assessed by a health-screening questionnaire. This questionnaire was administered to highlight any contraindications to taking part in the study. Exclusion criteria included those who had suffered a head injury, neurological disorder or neuro-developmental disorder. In addition, those who had any relevant food allergies or intolerances, smoked tobacco, drank excessive amounts of caffeine [>6 cups coffee/d (>450 mg caffeine/d)], or took illicit social drugs were also identified as contraindications to participation. All exclusion criteria were self-reported. The study was conducted in accordance with the Helsinki Declaration and ratified by the University's Research Ethics Committee. All enrolled participants provided written informed consent. This study was registered as a clinical trial with clinicaltrials.gov (NCT02381860).

Study Design

This study employed a placebo-controlled, double blinded, cross-over, randomised Latin square design with two experimental arms and a washout period of at least 14 days (mean ± SD, 15 ± 2 days); participants were randomly allocated to receive a 60 mL dose of a Montmorency cherry (MC) concentrate or a placebo (PLA). Fourteen participants received the MC concentrate on the first visit, with the remainder receiving the PLA. A washout of at least 14 days was chosen based on previous literature that suggests these phenolic compounds are quickly absorbed and/or excreted [3; 10; 36]. Each participant was required to attend the laboratory on three separate occasions. Each visit was at the same time of day (within participant) and was preceded by an overnight fast ($\geqslant$10 h). The first visit was an initial screening and familiarisation visit during which, participants were screened with

regards to the study exclusion/inclusion criteria, briefed with regards to compliance requirements, provided written informed consent and given full training and familiarisation on the cognitive tasks. On the subsequent experimental days, participants reported to the lab between 7 and 9am and a baseline blood pressure (BP) reading was taken. This was followed by a baseline cognitive assessment and cerebral blood flow measures by near infrared spectroscopy (NIRS) and transcranial Doppler (TCD). Participants then consumed the intervention beverage (either MC or PLA), following which, they sat quietly, watching one of a selection of non-arousing DVDs, during a 1 hour "absorption period". Subsequent cognitive assessments and blood flow measures were taken 1, 2, 3, and 5 h post consumption; BP was performed hourly. Between cognitive test sessions, participants continued to watch a selection of non-arousing DVDs. No additional food or fluid was provided during the study period except for low-nitrate mineral water, which was consumed *ad libitum*. The total volume of water consumed on the first experimental day was recorded and participants consumed the same volume on the second visit. The reason for this was to accurately examine the efficacy of the intervention. Previous studies have

Treatments and Dietary Control

A MC concentrate (CherryActive, Sunbury, UK) was stored at 4º C prior to use. Participants consumed either 60 mL of MC concentrate (which according to the manufacturer is estimated to be equivalent to ~180 whole cherries) or fruit-flavoured cordial in a double blind, cross-over manner. This estimate is based on the brix value of sucrose in 100 g of solution. The decision to use 60 mL was based on previous work that showed a greater uptake of anthocyanin and phenolic acids *in vivo* post-consumption when compared to a 30 mL dose [2; 3]. Additionally, this work identified that of the three Montmorency cherry analogue studied (frozen, dried and concentrated), the MC concentrate had the greatest antioxidant activity, total anthocyanin and phenolic content [3]. The MC concentrate was examined for total anthocyanins, total phenolic content and Trolox Equivalent Antioxidant Capacity using techniques previously described by Keane and colleagues [10]. The MC concentrate was found to contain 68.0 ±0.26 mg cyanidin-3-glucoside /L, 160.75 ± 0.55 mean gallic acid equiv/L and 0.59 ± 0.02 mean Trolox equiv/L, respectively. The concentrate was diluted with 100 mL of water prior to consumption.

The PLA supplement consisted of a commercially available, low fruit (<1%) cordial (Kia Ora, Coca Cola Enterprises, Uxbridge, UK) mixed with water, whey protein isolate (Arla Foods Ltd., Leeds, UK) and maltodextrin (MyProtein Ltd., Northwich, UK), to match the MC concentrate for volume and macronutrient content (Energy = 204 kcal, volume = 60 mL, carbohydrates = 49 g, protein = 2.2 g and fat = 0 g). The total anthocyanin content (used for colour purposes only) and total antioxidant capacity of the PLA were lower than the limits of detection, with trace amounts of phenolics (8.26 ± 0.04 mean gallic acid equiv/L). All drinks were prepared and all bottles were covered in tape prior to the study

by a third party. Prior to study commencement, it was explained to participants that the aim of the study was to investigate the effect of a fruit juice on vascular function; therefore they were unaware which beverage was the experimental drink. Participants were instructed to follow a low phenolic diet for 48 h prior to each arm of the trial by avoiding fruits, vegetables, tea, coffee, alcohol, chocolate, cereals, wholemeal bread, grains and spices and were asked to refrain from strenuous exercise. Compliance with the dietary restrictions was assessed with a standardised, self-reported 2-day dietary record. All participants complied with the low phenolic diet and this was confirmed via visual inspection of the food diaries.

Cognitive Tasks

All cognitive and mood measures 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. This assessment system has previously been shown to be sensitive to nutritional interventions following both acute [37] including acute supplementation with phenolics [27] and chronic supplementation [38]. At each of the aforementioned time points, a cognitive assessment test was completed. This assessment was a collection of three tasks that lasted 9 minutes; this was performed twice, which equated to 18 minutes in total. This was followed by a series of visual analogue scales to assess perceptions of fatigue and difficulty. The types of tests chosen have been previously used to detect changes in cognitive function following nutritional interventions [18; 27; 30]. In order to assess the relationship between specific brain regions and any changes in CBF, a selection of tasks that engender either higher or lower activation of the frontal cortex were employed. The "low activation" tasks comprised of a sustained attention test (digit vigilance). The "high activation" tasks (Rapid Visual Information Processing and Stroop tasks) entail a higher cognitive workload and have been shown to increase activity in the pre frontal cortex [39; 40]. The battery of cognitive tasks is described in more detail below.

Digit Vigilance

The DV task is a measure of sustained attention and psychomotor speed [41]. A single target digit was randomly selected and constantly displayed on the right hand side of the screen. A series of single digits appeared on the left hand side of the screen, one at a time, at the rate of 150 per minute. The participant was required to press the target button on the response pad as quickly as possible every time the digit in the series matched the target digit. The task lasted three minutes in total. Task outcomes included accuracy (%) and reaction time for correct responses (ms)

Rapid Visual Information Processing (RVIP)

The RVIP task is a measure of sustained attention and working memory [41]. This task requires the participant to monitor a continuous series of single digits for targets of three consecutive odd or three consecutive even digits. The digits are presented on the computer screen one at a time at the rate of 100 per minute in pseudo-random order, and the participant responds to the detection of a target string by pressing the target button on the response pad as quickly as possible. The task lasted three minutes in total. Task outcomes included number of target strings correctly detected (%) and average reaction time for correct detections (ms).

Stroop

The Stroop test is a measure of attention, inhibition and cognitive flexibility [42]. In this task, participants were presented with a colour name. The colour name presented was written in a coloured font, either the same "congruent" or a different "incongruent" font. Participants had to identify the colour of the font the word was written in, rather than the colour that the word was describing, via a response pad with coloured keys. Participants were presented with 90 stimuli in total taking ~3 minutes to complete. Task outcomes included number of correct responses (%) and the average response time for congruent and incongruent stimuli (ms).

Visual Analogue Scales

Participants were required to rate how "alert", "concentrated" and 'mentally fatigued' they felt and how 'difficult' they had found the tasks after each cognitive assessment repetition by indicating on a 100 mm line with the cursor ("not at all" at one end of the line and "extremely" at the other end) for alertness, fatigue and level of difficulty and ("very low" to "very high") for concentration. The VAS were scored as % along the line denoting more of the relevant adjective.

Blood Pressure

Blood pressure was measured using a non-invasive digital automatic BP monitor (M10-IT Omron Healthcare, UK). The BP cuff was fitted by the same researcher at each of the six time points. The inter- and intra-trial %CV for this method was 4.2 and 1.3% respectively.

Cerebrovascular Responses

Transcranial Doppler Imaging

Cerebral blood flow velocity in the middle cerebral artery (CBFV) was determined using transcranial Doppler sonography (Doppler-Box, Compumedics DWL, Singen, Germany). A 2 MHz Doppler probe was positioned over the right middle cerebral artery using previously described search techniques [43], and secured with an adjustable headset (DiaMon, Compumedics DWL). The mean depth for Doppler

signals was 62 ± 3 mm. All data were sampled at 200 Hz (PowerLab 16/30, ADInstruments Ltd, Oxfordshire, UK), and processed offline (LabChart version 5.4.2, ADInstruments Ltd).

Near Infrared Spectroscopy (NIRS)

The NIRS is a non-invasive brain imaging technique in which two nominal wavelengths of light, which are differentially absorbed by oxygenated (oxy-Hb) and deoxygenated haemoglobin (deoxy-Hb), respectively, are introduced through the skull via a laser emitter. They are then measured, following transit through the upper surface of the cortex, by an optode placed at a pre-set distance from the light source. NIRS has been used extensively as a technique for multiple-channel imaging of task-related brain activity over relevant areas of the head, including groups suffering from potential declines in CBF [(44)]. In the current study, cerebral oxygenation was assessed using near-infrared spectroscopy (NIRS; NIRO-200NX, Hamamatsu Photonics K.K., Japan). Two near-infrared sensors were placed over the left and right frontal lobe region of the forehead corresponding to the International 10–20 system Fp1 and Fp2 EEG positions; these signals were averaged to determine cerebral oxygenation. The sensors were secured to the skin using double-sided adhesive tape and shielded from ambient light using an elastic bandage. The sensors alternately emit two wavelengths of near-infrared light (≈765 and 855 nm) with an emitter/optode separation distance of 4 cm. The NIRS data were acquired continuously and output every 5 s and recorded for later offline analysis. The NIRS data output was time stamped at the start of each task segment to assure that data corresponded to the relevant period of task performance. Relative concentration changes in Oxy-Hb, Deoxy-Hb and Total-Hb were calculated.

Statistical Analysis

Cognitive performance, BP and CBFV data were analysed by using a treatment × time point mixed model analysis of variance (ANOVA). Maulchy's Test of Sphericity was used to check homogeneity of variance for all ANOVA analyses; where necessary, violations of the assumption were corrected using the Greenhouse–Geisser adjustment. Significant main effects were followed up using Šidák *post hoc* analysis. The analysis of NIRS data was conducted with Minitab 15 for Windows (Minitab Inc, State College, PA). Prior to the primary analysis, a within subjects Analysis of Variance (ANOVA) was carried out with left/right optode included as a factor (hemisphere x treatment group) for each task. As there were no treatment related interactions involving hemisphere the data from the 2 channels were averaged across hemispheres for the analysis and figures reported below. For each variable (oxy-Hb, deoxy-Hb and total Hb), data were converted to "change from baseline" (calculated from baseline pre-treatment period). Task length was fixed for the DV (180 s) and RVIP (180 s), but NIRS data from the Stroop test were truncated so that the same amount of data was analysed for all participants during each task period. Data from the 'resting/absorption' period (minutes 1-60) and the

task performance were analysed separately for all time points [pre–supplement, 1, 2, 3 and 5 h]. Data from the 'resting/absorption' period was averaged across 6 equal 10-min epochs and analysed by two – way repeated measures analysis of variance (ANOVA) (epoch × treatment). Data from the task period data was averaged across 6 equal 3-min epochs. This data was analysed by a three-way repeated measures ANOVA (task (epoch) × treatment × time point).

In the absence of any directly relevant data, it was suggested that a sample size of twenty – four would be adequate to have greater than an 80% chance of detecting the medium effect sizes demonstrated in previous research assessing the effect of polyphenols on NIRS parameters [45]. The resultant sample size of 27 (for a within-subjects, crossover design) was in excess of the typical sample sizes for NIRS investigations.

Results

Thirty male and female participants volunteered to take part in the study, but three participants voluntarily withdrew after the first study day due to time constraints (n=27). There were no adverse events reported in response to the intervention products. All participants complied with the low-polyphenolic diet according to the food diaries.

Cognitive performance and mood

No significant treatment-related differences were observed for any of the cognitive or mood measures ($p>0.05$). The absolute values for task scores and mood ratings are given in Tables 1 and 2, respectively.

Blood pressure

Systolic blood pressure (SBP) exhibited a time ($p \leq 0.01$), and treatment × time interaction effects ($p=0.002$). A post-hoc Šidák test indicated that this difference occurred at 1, 2, 3 h post supplementation in the MC group, with peak reductions from baseline in postprandial SBP of 6 ± 2 mmHg at 1 h post MC consumption (Figure 1). There was no time, treatment or treatment × time interaction effects observed for diastolic blood pressure (DBP).

Transcranial Doppler Imaging

There was no time, treatment or treatment × time interaction effects observed for cerebral blood flow velocity ($p>0.05$).

Near-IR spectroscopy parameters

Oxygenated haemoglobin (oxy-Hb). Similarly, there was a significant interaction between treatment and posttreatment epoch on the initial ANOVA during the resting/absorption period ($p=0.029$).

299 Reference to planned comparisons showed that there were significantly higher oxy-Hb concentrations
300 during the 30-40 min epoch of the resting/absorption period for MC concentrate. MC concentrate
301 also resulted in higher oxy-Hb concentrations during each epoch of task performance 1 h post
302 consumption ($p=0.019$). Thereafter, there were no significant differences in oxy-Hb ($p>0.05$) (Figure
303 2A).

304 *Deoxygenated haemoglobin (deoxy – Hb*). There were no significant differences in terms of deoxy-Hb
305 during either the resting/absorption or task performance periods ($p>0.05$).

306 Total haemoglobin (Total-Hb). There was no significant interaction between treatment and
307 posttreatment epoch on the initial ANOVA during the resting/absorption period ($p > 0.05$). MC
308 concentrate resulted in higher total-Hb concentrations during each epoch of task performance 1 h post
309 consumption ($p \leqslant 0.01$). Thereafter, there were no significant differences in total-Hb ($p>0.05$)
310 (Figure 2B).

311 *Task-related differences.* There were no significant differences seen in the hemodynamic response to
312 the DV, RVIP or Stroop tasks.

313

Discussion

To the best of our knowledge, this study was the first to investigate the acute effects of Montmorency tart cherries consumption on cerebral blood flow variables and cognitive performance in a middle aged population. In support of our hypothesis, this study presents new information that in comparison to placebo, the consumption of a MC concentrate resulted in acute modulation of CBF parameters in the frontal cortex during task performance as indicated by the elevated concentration of total-Hb, with an identical pattern observed with oxy-Hb. This effect was evident for the cognitive assessment 1 h post MC consumption. These CBF observations were not associated with any significant modulation of cognitive performance or mood. There was also a significant reduction in SBP for up to three hours post MC consumption relative to the placebo.

Compromised cerebral blood flow has been suggested as a key contributor to cognitive function decline observed with advancing age and in a number of neurodegenerative diseases [46]. The results of the current study demonstrate that MC concentrate can modulate aspects of brain function, which this was evident 1 h post consumption. Total-Hb and oxy-Hb were increased toward the end of the 60-min resting/absorption period, although not significantly in most cases, and during the cognitive assessment 1 h post consumption. However, there were no concomitant changes in deoxy-Hb across any of the time points. These results are consistent with previous studies using compounds and whole foods to demonstrate a positive effect on cognitive function and CBF. Kennedy and colleagues [30] demonstrated an increase in total-Hb and oxy-Hb following single doses of orally administered resveratrol and more recently, Wightman et al. [31] following beetroot juice ingestion. In both of these studies, total-Hb was increased during the first epoch of task performance. However, whilst total-Hb remained higher in the resveratrol study throughout the 40 minute cognitive assessment, it was decreased during the last 5 repetitions when participants were supplemented with beetroot juice. In the current study, no significant differences were seen following the first task period. The limitations associated with NIRS have consistently been highlighted [47], and in the past few years, fMRI and other neuroimaging techniques have been used to assess the effect of a nutritional supplement on CBF [33; 48].

In terms of higher total-Hb concentrations, the modulation seen in the current study may be due to the vasorelaxatory and antihypertensive properties of some of the phenolic acids (vanillic and protocatechuic acid) contained in the MC concentrate [3; 10]. The time points (~1 hour post) at which these metabolites are seen in the plasma coincide with improvements in vascular function [10], and modulation in CBF in the current study. Although, deoxy-Hb was not modulated by the experimental beverage, it should be noted that there was a trend for a reduced concentration throughout the task period. Furthermore, in the current study, task had no significant effect on cerebral modulation. It has previously been speculated that “high activation” tasks such as RVIP result in a higher increase in

CBF than does performance in "low activation" tasks, for example digit vigilance. This can be largely attributed to the relative cognitive demands of the two tasks, with RVIP requiring the monitoring of rapidly changing digits along with a passive contribution from working memory. We speculate that these very early on effects on CBF in the current study are more likely to be associated with the sensory properties of the MC concentrate as previous studies have showed demonstrated that a number of sensory factors including differing taste and flavours are likely to modulate frontal cortex activity [49; 50]. Marciani and colleagues previously demonstrated that several brain areas were activated immediately after swallowing particularly when supplements had a strong (combined) taste or aroma. It could be argued that the MC concentrate was more sensory stimulating than the placebo. However, a full analysis of sensory properties was outside the remit of the current study.

Although the current study highlights an acute heightened NIRS response in brain regions responsible for task performance, there no was effect on cognitive performance. Supplementary oxygen [51] has been shown to positively influence cognitive performance in a healthy population. Therefore, it makes the expectation tenable that increases in CBF could be beneficial to acute cognitive performance via increasing the delivery of oxygenated blood metabolic substrate to, and efflux of metabolites from the brain, which is critical for brain function [52]. Importantly, despite some indication of improved blood flow, the current study showed no changes in cognitive task performance between experimental conditions. Nevertheless, these results do not stand alone; Caldwell et al. [24] previously demonstrated that regardless of dose, cherry juice had no acute impact on cognitive function in young people, older people or dementia patients. They concluded that although cherry juice may have an acute impact on cardiovascular function, there was no change in cognitive performance 6 h post consumption. However, Caldwell and colleagues used sweet cherries as an intervention, it has been speculated that sweet cherries are not as rich in phytochemical compounds as tart/sour cherries [6]. Furthermore, cognitive assessments and blood flow measures were taken at baseline, 2 and 6 h post consumption, the current study attempted to explore the time points following consumption in more detail (hourly), however it is possible that any potential changes might still have been missed. Contrastingly, a chronic study by the same group [25] reported that the daily consumption of sweet cherries for 12 weeks improved cognitive performance across almost all tasks in older adults with mild-to-moderate dementia; this group showed improvements for category verbal fluency and tasks relating to verbal learning and memory and concluded the positive changes have clinical relevance for these cognitive improvements. Therefore, it is likely that regulation of blood flow and cognition are extremely complex, with multiple overlapping regulatory mechanisms paradigms and contributing structural components [53], and therefore more likely to be influenced by chronic supplementation. This also accords with previous observations in similar trials, where Kelly et al. [54] and Thompson et al. [55] showed that after acute beetroot supplementation, there were no changes in cognitive performance for concentration, memory, attention or information

processing ability. However, when older type 2 diabetics were supplemented with beetroot juice for 14 days, they experienced a significant improvement in simple reaction time compared to a control group [56]. These somewhat contrasting results may be partly explained by dose duration. It would seem that the cerebrovascular response required to elicit measurable changes in cognitive function can only be achieved with longer term dosing strategies that have the potential to induce sustained modifications to cerebrovascular function [26]. However, contrary to this suggestion, two recent additions to the literature have demonstrated positive effects on cognitive function following acute blackcurrant [27] and wild blueberries (WBB) [28] supplementation. Acute blackcurrant supplementation was shown to improve RVIP accuracy and reaction time on the DV task in healthy people. Whilst, Whyte and colleagues demonstrated that acute cognitive benefits can be observed in 7-10-year old-children with an anthocyanin-rich blueberry intervention. The Page's test revealed the consistency and strength of this finding with WBB supplementation leading to significant overall improvements in cognition function, with the best change from baseline performance associated with 30g WBB treatment, intermediate performance with the 15g WBB treatment, and least effective performance with the vehicle treatment. Given that the protective cognitive effect of fruits is attributed to their high anthocyanin content, the anthocyanin dose in both of these studies was marginally higher than the current investigation (253 mg and 552 mg vs. 68 mg cyanidin-3-glucoside /L), this might go some way in explaining the inconsistent findings. It is also worthy to note, the cognitive tasks in the current study were selected on the basis of previous sensitivity to nutritional interventions [18; 27], however, these tasks may not be adequately sensitive to detect change in acute studies as perhaps this particular intervention could affect different cognitive domains (i.e. memory, recall). The most important consideration in setting up a suitable framework for measuring human cognitive function in polyphenol or flavonoid research is to determine methods that are sensitive to dietary changes and repeatable over time, simple to interpret and specific to cognitive domains [57]. Furthermore, all participants in the current study were healthy with no apparent issues pertaining to cerebral blood flow or cognitive ability. It is logical to question if that could mean that sufficient blood flow already exist for maximal cognitive performance and therefore, increasing blood flow beyond this threshold does not have any acute benefits on cognitive performance.

Additionally, there was no effect of the intervention on mood. This is somewhat surprising as mental fatigue has been previously shown to be receptive to cocoa flavanols in healthy adults [18]. However, Scholey and colleagues employed repeated 10-min cycles of a Cognitive Demand Battery (two serial subtraction tasks [Serial Threes and Serial Sevens] and RVIP), over the course of 1 h. Therefore, the cognitive assessment adopted in the current study might not have been as taxing on the brain as the aforementioned CDB with very limited rest time between repetitions.

There was a significant decrease in systolic blood pressure following MC supplementation when compared to placebo. These findings are in agreement with a previous study that reported a positive

421 modulation of SBP in early hypertensive males following MC ingestion [10]. This is not surprising, as
422 participants in the current study had moderately elevated systolic blood pressure above the published
423 ideal values at baseline - 128/82 mmHg. Kapil et al. [58] noted that the magnitude of change in the BP
424 response is directly related to baseline BP therefore, those who have a higher BP, will likely
425 experience a greater change following an intervention. The current study is particularly noteworthy as
426 data from prospective, observational studies have shown a reduction in mean SBP of 5-6 mmHg over
427 a five year period was associated with 38% and 23% reduced risk of stroke and coronary heart
428 disease, respectively [59]. Here, we reported peak reductions in postprandial SBP of 6 ± 2 mmHg
429 relative to the placebo. This finding, along with the modulation of CBF 1 h post MC consumption
430 supports the growing body of evidence showing an inverse association between the risk of chronic
431 human diseases and the consumption of polyphenolic rich diet [60; 61].

432 The findings of the current study should be interpreted with a certain degree of caution because of the
433 dietary restrictions imposed on participants. It is extremely unlikely that one would consume a diet
434 that is free from polyphenol-rich foods and as a result, future work should attempt to demonstrate
435 synergistic effects of MC supplementation within habitual dietary practices. Furthermore, a digital
436 automatic BP monitor was used in the current study. The accuracy of this method has been called
437 into question [62]. Future studies should consider using ambulatory blood pressure measurements,
438 where readings are taking at regular intervals. Many studies have now confirmed that blood pressure
439 measured over a 24-hour period is superior to clinic blood pressure in predicting future cardiovascular
440 events [63]. A timeframe of 5 h was utilized based on previous findings that phenolic compounds are
441 quickly absorbed and/or excreted [3; 36] and that any positive effects in vascular function are transient
442 and return to baseline after four hours [10]. However, it is possible that this timeframe may not long
443 enough to capture the absorption of potentially other bioactive phenolic compounds provided by
444 cherries in the colon.

445 In summary, the findings from this study suggest that MC concentrate can acutely modulate CBF in
446 the prefrontal cortex characterized by increased concentrations of both total-Hb and oxy-Hb. Despite
447 this evident modulation, these results do not translate to improvements in cognition or mood in the
448 hours following consumption. Finally, this study reaffirms previous findings that demonstrate a
449 significant improvement in SBP following MC supplementation.

450 **Acknowledgements**

451 The Cherry Research Committee of the Cherry Marketing Institute (Lansing, MI, USA), a not
452 for profit organisation, provided support for a PhD studentship associated with this work. All
453 other elements of the study were funded by Northumbria University. The funders had no role
454 in the study design, data collection and analysis, decision to publish, or preparation of the

manuscript. The authors declare no conflict of interest. The authors would like to thank their participants in this investigation. The authors' responsibilities were as follows: K.K., R.C.V., C.H. and G.H. designed the study; K.K. conducted the research; K.K. G.H and C.H. analysed and interpreted the data; K.K., C.H. and G.H. wrote the paper; G.H. and K.M.K. had primary responsibility for final content. All authors read and approved the final manuscript. The authors declare no conflict of interest.

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Table 1. Effects of MC concentrate and PLA on various aspects of cognitive performance in healthy, middle aged adults.

		Task battery repetition												
		Baseline		1		2		3		5		ANOVA		
Measures	Treatment	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Effect	*F*	*P*
DV (%)	60 mL MC	94.20	1.11	94.30	1.40	93.58	1.38	92.29	1.67	92.28	2.00	T	0.087	0.771
	Placebo	95.17	0.88	94.47	1.16	94.08	1.33	92.59	1.78	92.10	2.02	T × R	0.137	0.890
DV RT (ms)	60 mL MC	455.41	8.08	461.85	8.99	464.01	8.01	472.10	8.93	470.05	9.35	T	3.793	0.062
	Placebo	455.48	8.19	461.02	8.58	465.76	9.10	454.59	11.06	443.96	16.15	T × R	2.109	0.135
RVIP (%)	60 mL MC	53.40	5.04	52.85	4.23	51.24	5.08	51.70	4.98	52.31	4.80	T	0.027	0.870
	Placebo	51.69	4.30	55.12	4.67	51.34	4.87	53.47	4.47	52.15	4.73	T × R	0.391	0.759
RVIP RT(ms)	60 mL MC	491.55	32.22	517.05	12.13	522.39	10.99	527.96	10.99	504.35	11.81	T	0.269	0.608
	Placebo	526.14	11.55	520.01	12.02	483.81	29.07	505.52	17.11	506.96	15.74	T × R	1.145	0.316
Stroop (%)	60 mL MC	98.65	0.24	98.78	0.26	98.69	0.24	98.77	0.23	98.62	0.25	T	0.960	0.414
	Placebo	98.58	0.24	98.65	0.30	98.89	0.24	99.01	0.20	98.96	0.21	T × R	0.667	0.298
Stroop RT (ms)	60 mL MC	789.04	30.74	774.62	26.85	778.11	36.00	753.95	26.71	761.07	24.48	T	0.214	0.648
	Placebo	814.87	37.53	764.02	29.23	764.00	29.23	763.36	29.75	805.29	68.20	T × R	0.677	0.487

All values are means ± SEM (n=27) T, treatment; R, repetition; DV, digit vigilance; RVIP, rapid visual information processing; RT, reaction time.

Table 2. Effects of MC concentrate and PLA on mood in healthy, middle aged subjects.

		Task battery repetition												
		Baseline		1		2		3		5		ANOVA		
Measures	Treatment	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Effect	*F*	*P*
Alert	60 mL MC	35.67	4.55	35.39	3.55	40.31	4.11	42.91	4.41	45.22	4.44	T	0.415	0.525
	Placebo	35.10	3.91	34.76	3.49	43.13	3.70	45.30	3.94	49.85	4.37	T × R	0.763	0.477
Concentration	60 mL MC	57.19	4.83	52.50	3.68	52.39	3.69	50.94	3.49	47.96	3.42	T	0.287	0.597
	Placebo	51.75	3.61	57.02	3.19	48.80	3.44	49.15	3.50	46.93	3.52	T × R	1.417	0.250
Mental fatigue	60 mL MC	60.74	4.29	61.54	3.76	60.94	4.13	58.69	3.97	57.20	4.21	T	0.163	0.690
	Placebo	61.65	3.95	63.44	3.32	55.76	3.82	54.30	4.09	56.85	4.08	T × R	1.281	0.288
Difficulty	60 mL MC	38.41	3.91	38.94	3.46	40.50	4.21	41.57	4.21	44.59	3.81	T	0.014	0.907
	Placebo	40.73	3.69	36.65	3.41	39.78	3.08	38.96	3.43	44.96	3.50	T × R	0.631	0.579

All values are means ± SEM (n=27) T, treatment; R, repetition.

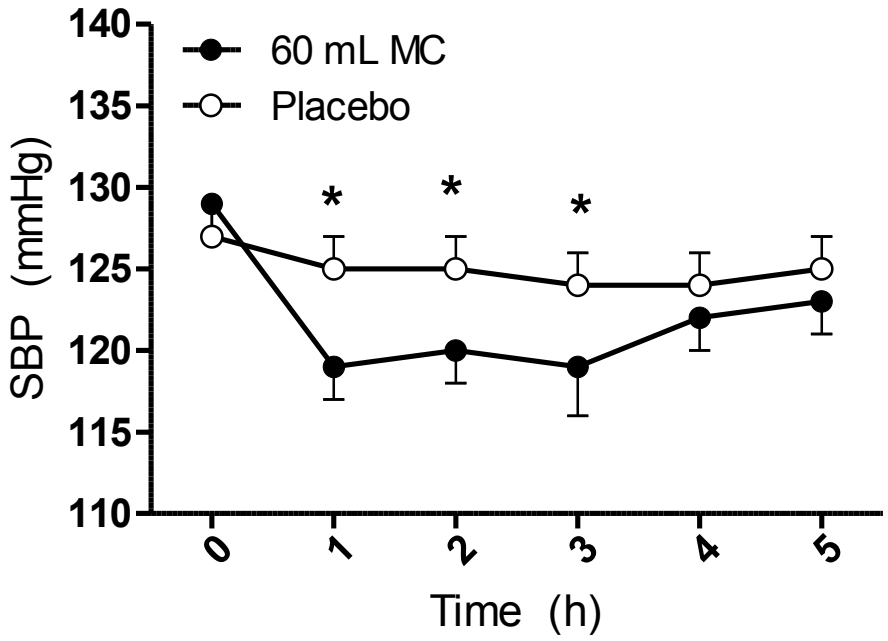

Figure 1: Time course of systolic blood pressure (mean ± SEM) response after consumption of MC concentrate- and macronutrient – matched control (n=27). Significantly different from the placebo drink: * $p < 0.05$

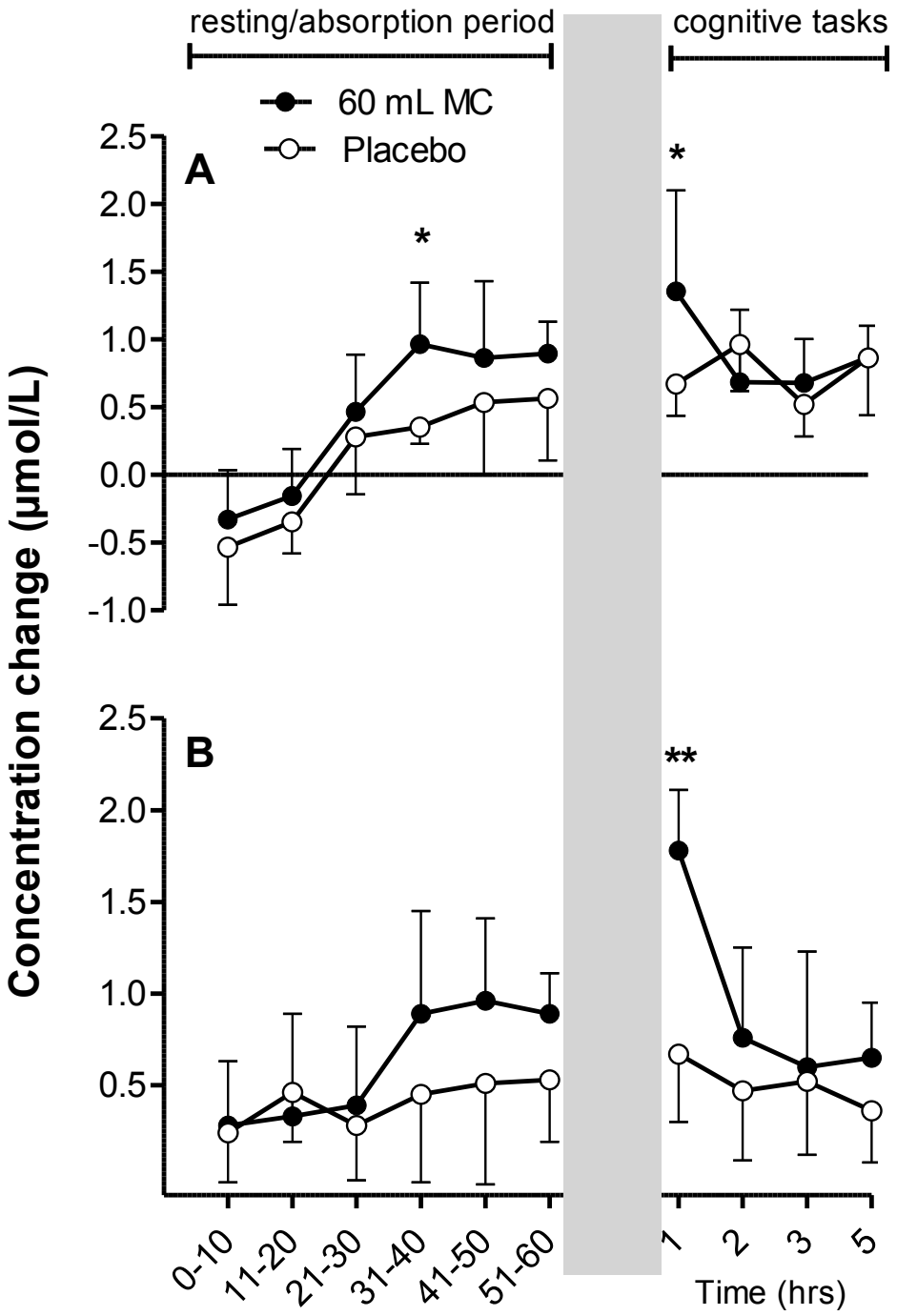

Figure 2: A: Mean (± SEM) changes in concentrations of oxy-haemoglobin and B: total haemoglobin during a 60-min absorption period and subsequent cognitive task assessments 1, 2, 3 and 5 h post 60mL MC concentrate or placebo. Significantly different from the placebo: * $p < 0.05$, ** $p < 0.01$.