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Citation: Keane, Karen, Veasey, Rachel, Haskell, Crystal and Howatson, Glyn (2016) Tart Montmorency cherries (*Prunus Cerasus* L.) modulate vascular function acutely, in the absence of improvement in cognitive performance. *British Journal of Nutrition*, 116 (11). pp. 1935-1944. ISSN 0007-1145

Published by: Cambridge University Press

URL: <https://doi.org/10.1017/S0007114516004177>
<<https://doi.org/10.1017/S0007114516004177>>

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

Keane, KM¹, Haskell-Ramsay, CF², Veasey, RC² 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

1 **Abstract**

2 Cerebral blood volume and metabolism of oxygen declines as part of human ageing and this has been
3 previously shown to be related to cognitive decline. There is some evidence to suggest that
4 polyphenol-rich foods can play an important role in delaying the onset or halting the progression of
5 age-related health disorders such as cardiovascular and Alzheimer's disease, and to improve cognitive
6 function. In the present study, an acute, placebo-controlled, double blinded, cross-over, randomised
7 Latin square design study with a wash-out period of at least 14 days was conducted in twenty-seven
8 middle aged (defined as 45-60 years) volunteers. Participants received either a 60 mL dose of a
9 Montmorency tart cherry concentrate (MC), which contains 68.0 ± 0.26 mg cyanidin-3-glucoside /L,
10 160.75 ± 0.55 mean gallic acid equiv/L and 0.59 ± 0.02 mean Trolox equiv/L, respectively or a
11 placebo (PLA). Cerebrovascular responses, cognitive performance and blood pressure were assessed
12 at baseline and 1, 2, 3 and 5 h following consumption. There were significant differences in
13 concentrations of total and oxy-haemoglobin during the task period 1 h post MC consumption ($p \leq$
14 0.05). Furthermore, MC consumption significantly lowered SBP ($p \leq 0.05$) over a period of 3 h, with
15 peak reductions of 6 ± 2 mmHg at 1 h post MC consumption relative to the placebo. Cognitive
16 function and mood were not affected. These results show that a single dose of MC concentrate can
17 modulate certain variables of vascular function; however this does not translate to improvements in
18 cognition or mood.

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31 **Tart Montmorency cherries modulate vascular function acutely, in the absence of improvement** 32 **in cognitive function**

33 **Introduction**

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

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

61 Seymour et al ⁽²¹⁾ showed that intake of 1% tart cherry diet significantly reduced stroke-related
62 phenotypes in rats. Tart cherry intake also reduced brain NFκB activity and the related pro-
63 inflammatory transcripts. Interestingly in 2015, Kirakosyan and colleagues ⁽²²⁾ confirmed that tart
64 cherry anthocyanins cross the blood-brain barrier. In a more recent addition to the literature, thirty
65 19-month-old male Fischer 344 rats who received either a control diet or a diet supplemented with 2%

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

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

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

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

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

111 **Methods**

112 **Participants**

113 Thirty healthy middle aged (defined as 45-60 years) adults (10 female, 20 male, 28 right-handed, 2
114 left-handed) were recruited to take part in the study; the mean \pm SD age, stature, mass and BMI were
115 50 ± 6 years, 170.7 ± 9.1 cm, 76.0 ± 16.0 kg and 26.1 ± 4.9 kg/m², respectively. All participants were
116 in apparent good health as assessed by a health-screening questionnaire. This questionnaire was
117 administered to highlight any contraindications to taking part in the study. Exclusion criteria included
118 those who had suffered a head injury, neurological disorder or neuro-developmental disorder. In
119 addition, those who had any relevant food allergies or intolerances, smoked tobacco, drank excessive
120 amounts of caffeine [>6 cups coffee/d (>450 mg caffeine/d)], or took illicit social drugs were also
121 identified as contraindications to participation. All exclusion criteria were self-reported. The study
122 was conducted in accordance with the Helsinki Declaration and ratified by the University's Research
123 Ethics Committee. All enrolled participants provided written informed consent. This study was
124 registered as a clinical trial with clinicaltrials.gov (NCT02381860).

125 **Study Design**

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

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

149 **Treatments and Dietary Control**

150 A MC concentrate (CherryActive, Sunbury, UK) was stored at 4° C prior to use. Participants
151 consumed either 60 mL of MC concentrate (which according to the manufacturer is estimated to be
152 equivalent to ~180 whole cherries) or fruit-flavoured cordial in a double blind, cross-over manner.
153 This estimate is based on the brix value of sucrose in 100 g of solution. The decision to use 60 mL
154 was based on previous work that showed a greater uptake of anthocyanin and phenolic acids *in vivo*
155 post-consumption when compared to a 30 mL dose^(2; 3). Additionally, this work identified that of the
156 three Montmorency cherry analogue studied (frozen, dried and concentrated), the MC concentrate had
157 the greatest antioxidant activity, total anthocyanin and phenolic content⁽³⁾. The MC concentrate was
158 examined for total anthocyanins, total phenolic content and Trolox Equivalent Antioxidant Capacity
159 using techniques previously described by Keane and colleagues⁽¹⁰⁾. The MC concentrate was found
160 to contain 68.0 ± 0.26 mg cyanidin-3-glucoside /L, 160.75 ± 0.55 mean gallic acid equiv/L and 0.59 ±
161 0.02 mean Trolox equiv/L, respectively. The concentrate was diluted with 100 mL of water prior to
162 consumption.

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

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

178 **Cognitive Tasks**

179 All cognitive and mood measures were delivered using the Computerised Mental Performance
180 Assessment System (COMPASS, Northumbria University, Newcastle upon Tyne, UK), a purpose-
181 designed software application for the flexible delivery of randomly generated parallel versions of
182 standard and novel cognitive assessment tasks. This assessment system has previously been shown to
183 be sensitive to nutritional interventions following both acute⁽³⁷⁾ including acute supplementation with
184 phenolics⁽²⁷⁾ and chronic supplementation⁽³⁸⁾. At each of the aforementioned time points, a cognitive
185 assessment test was completed. This assessment was a collection of three tasks that lasted 9 minutes;
186 this was performed twice, which equated to 18 minutes in total. This was followed by a series of
187 visual analogue scales to assess perceptions of fatigue and difficulty. The types of tests chosen have
188 been previously used to detect changes in cognitive function following nutritional interventions^{(18; 27;}
189³⁰⁾. In order to assess the relationship between specific brain regions and any changes in CBF, a
190 selection of tasks that engender either higher or lower activation of the frontal cortex were employed.
191 The “low activation” tasks comprised of a sustained attention test (digit vigilance). The “high
192 activation” tasks (Rapid Visual Information Processing and Stroop tasks) entail a higher cognitive
193 workload and have been shown to increase activity in the pre frontal cortex^(39; 40). The battery of
194 cognitive tasks is described in more detail below.

195 *Digit Vigilance*

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

202 *Rapid Visual Information Processing (RVIP)*

203 The RVIP task is a measure of sustained attention and working memory ⁽⁴¹⁾. This task requires the
204 participant to monitor a continuous series of single digits for targets of three consecutive odd or three
205 consecutive even digits. The digits are presented on the computer screen one at a time at the rate of
206 100 per minute in pseudo-random order, and the participant responds to the detection of a target string
207 by pressing the target button on the response pad as quickly as possible. The task lasted three minutes
208 in total. Task outcomes included number of target strings correctly detected (%) and average reaction
209 time for correct detections (ms).

210 *Stroop*

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

218 *Visual Analogue Scales*

219 Participants were required to rate how “alert”, “concentrated” and ‘mentally fatigued’ they felt and
220 how ‘difficult’ they had found the tasks after each cognitive assessment repetition by indicating on a
221 100 mm line with the cursor (“not at all” at one end of the line and “extremely” at the other end) for
222 alertness, fatigue and level of difficulty and (“very low” to “very high”) for concentration. The VAS
223 were scored as % along the line denoting more of the relevant adjective.

224 **Blood Pressure**

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

228 **Cerebrovascular Responses**

229 **Transcranial Doppler Imaging**

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

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

236 **Near Infrared Spectroscopy (NIRS)**

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

254 **Statistical Analysis**

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

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

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

278 **Results**

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

283 **Cognitive performance and mood**

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

287 **Blood pressure**

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

293 **Transcranial Doppler Imaging**

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

296 **Near-IR spectroscopy parameters**

297 *Oxygenated haemoglobin (oxy-Hb)*. Similarly, there was a significant interaction between treatment
298 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 ($p \leq 0.01$). 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

314 **Discussion**

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

324 Compromised cerebral blood flow has been suggested as a key contributor to cognitive function
325 decline observed with advancing age and in a number of neurodegenerative diseases ⁽⁴⁶⁾. The results
326 of the current study demonstrate that MC concentrate can modulate aspects of brain function, which
327 this was evident 1 h post consumption. Total-Hb and oxy-Hb were increased toward the end of the
328 60-min resting/absorption period, although not significantly in most cases, and during the cognitive
329 assessment 1 h post consumption. However, there were no concomitant changes in deoxy-Hb across
330 any of the time points. These results are consistent with previous studies using compounds and whole
331 foods to demonstrate a positive effect on cognitive function and CBF. Kennedy and colleagues ⁽³⁰⁾
332 demonstrated an increase in total-Hb and oxy-Hb following single doses of orally administered
333 resveratrol and more recently, Wightman et al. ⁽³¹⁾ following beetroot juice ingestion. In both of these
334 studies, total-Hb was increased during the first epoch of task performance. However, whilst total-Hb
335 remained higher in the resveratrol study throughout the 40 minute cognitive assessment, it was
336 decreased during the last 5 repetitions when participants were supplemented with beetroot juice. In
337 the current study, no significant differences were seen following the first task period. The limitations
338 associated with NIRS have consistently been highlighted ⁽⁴⁷⁾, and in the past few years, fMRI and
339 other neuroimaging techniques have been used to assess the effect of a nutritional supplement on CBF
340 ^(33; 48).

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

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

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

385 processing ability. However, when older type 2 diabetics were supplemented with beetroot juice for
386 14 days, they experienced a significant improvement in simple reaction time compared to a control
387 group ⁽⁵⁶⁾. These somewhat contrasting results may be partly explained by dose duration. It would
388 seem that the cerebrovascular response required to elicit measurable changes in cognitive function can
389 only be achieved with longer term dosing strategies that have the potential to induce sustained
390 modifications to cerebrovascular function ⁽²⁶⁾. However, contrary to this suggestion, two recent
391 additions to the literature have demonstrated positive effects on cognitive function following acute
392 blackcurrant ⁽²⁷⁾ and wild blueberries (WBB) ⁽²⁸⁾ supplementation. Acute blackcurrant
393 supplementation was shown to improve RVIP accuracy and reaction time on the DV task in healthy
394 people. Whilst, Whyte and colleagues demonstrated that acute cognitive benefits can be observed in
395 7-10-year old-children with an anthocyanin-rich blueberry intervention. The Page's test revealed the
396 consistency and strength of this finding with WBB supplementation leading to significant overall
397 improvements in cognition function, with the best change from baseline performance associated with
398 30g WBB treatment, intermediate performance with the 15g WBB treatment, and least effective
399 performance with the vehicle treatment. Given that the protective cognitive effect of fruits is
400 attributed to their high anthocyanin content, the anthocyanin dose in both of these studies was
401 marginally higher than the current investigation (253 mg and 552 mg vs. 68 mg cyanidin-3-glucoside
402 /L), this might go some way in explaining the inconsistent findings. It is also worthy to note, the
403 cognitive tasks in the current study were selected on the basis of previous sensitivity to nutritional
404 interventions ^(18: 27), however, these tasks may not be adequately sensitive to detect change in acute
405 studies as perhaps this particular intervention could affect different cognitive domains (i.e. memory,
406 recall). The most important consideration in setting up a suitable framework for measuring human
407 cognitive function in polyphenol or flavonoid research is to determine methods that are sensitive to
408 dietary changes and repeatable over time, simple to interpret and specific to cognitive domains ⁽⁵⁷⁾.
409 Furthermore, all participants in the current study were healthy with no apparent issues pertaining to
410 cerebral blood flow or cognitive ability. It is logical to question if that could mean that sufficient
411 blood flow already exist for maximal cognitive performance and therefore, increasing blood flow
412 beyond this threshold does not have any acute benefits on cognitive performance.

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

419 There was a significant decrease in systolic blood pressure following MC supplementation when
420 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 taken 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

455 manuscript. The authors declare no conflict of interest. The authors would like to thank their
456 participants in this investigation. The authors' responsibilities were as follows: K.K., R.C.V.,
457 C.H. and G.H. designed the study; K.K. conducted the research; K.K. G.H and C.H. analysed
458 and interpreted the data; K.K., C.H. and G.H. wrote the paper; G.H. and K.M.K. had primary
459 responsibility for final content. All authors read and approved the final manuscript. The
460 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.

Measures	Treatment	Task battery repetition										ANOVA		
		Baseline		1		2		3		5		Effect	<i>F</i>	<i>P</i>
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.

Measures	Treatment	Task battery repetition										ANOVA		
		Baseline		1		2		3		5		Effect	<i>F</i>	<i>P</i>
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.

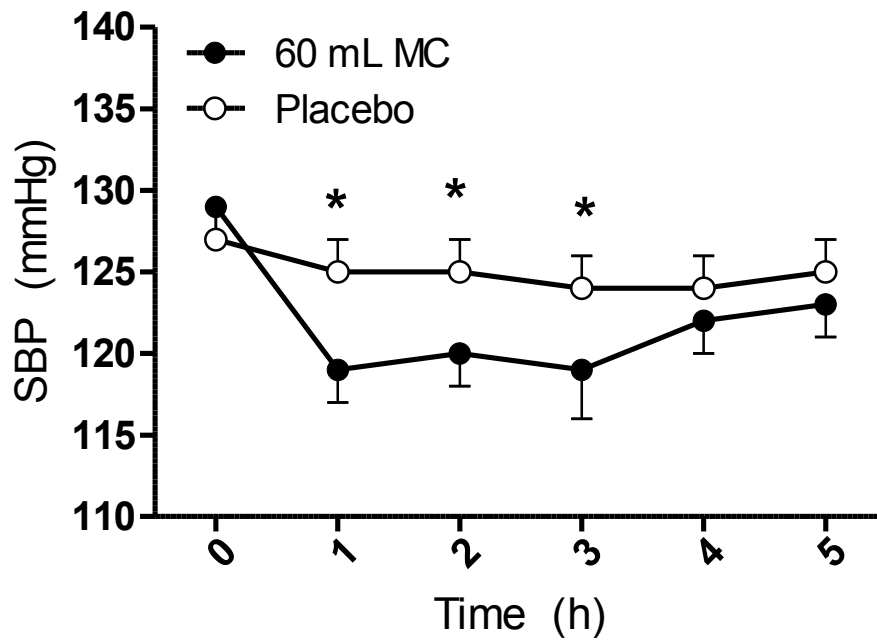


Figure 1: Time course of systolic blood pressure (mean \pm SEM) response after consumption of MC concentrate- and macronutrient – matched control (n=27). Significantly different from the placebo drink: * $p < 0.05$

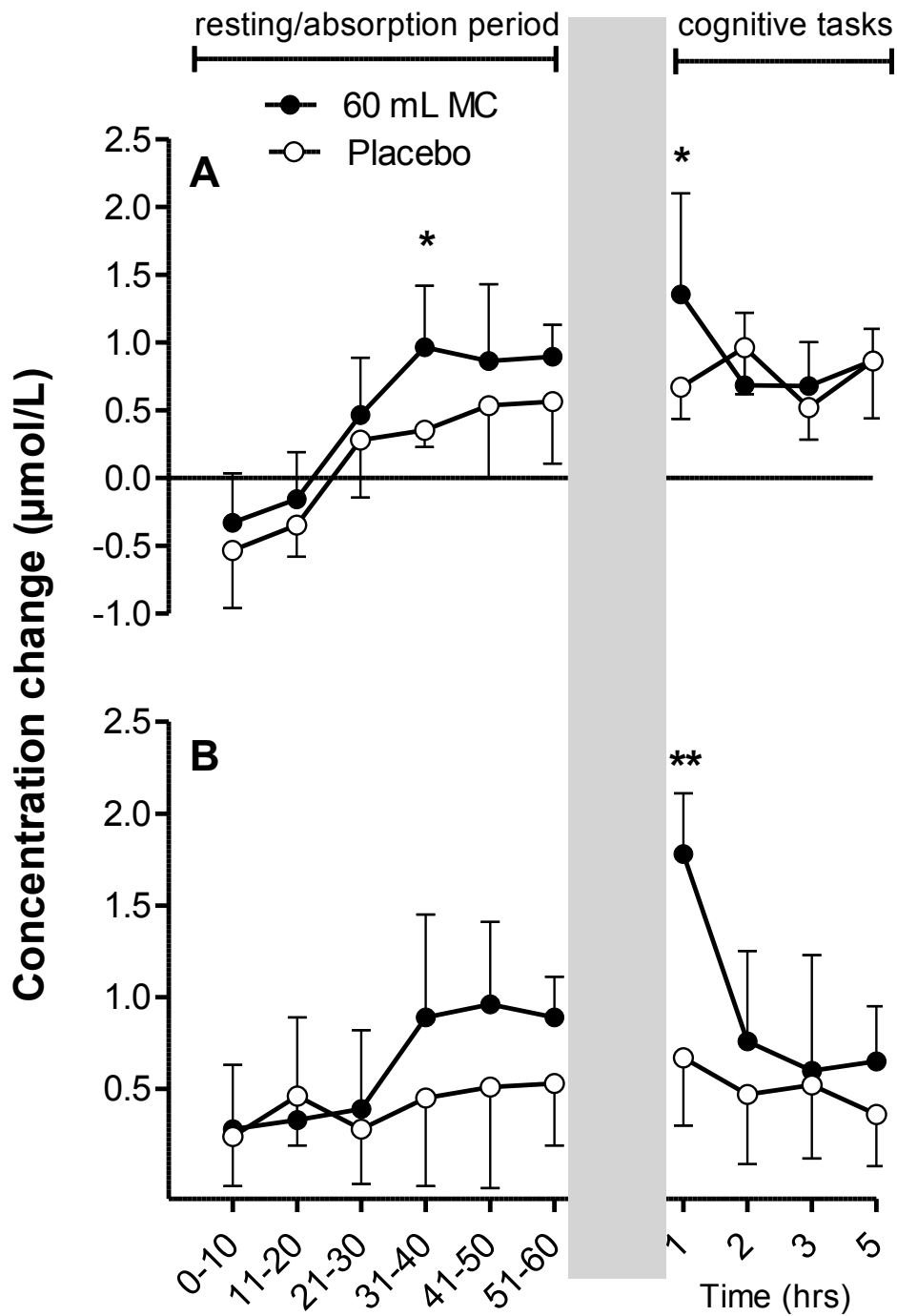


Figure 2: A: Mean (\pm SEM) changes in concentrations of oxy-haemoglobin and B: total haemoglobin during a 60-min absorption period and subsequent cognitive task assessments 1, 2, 3 and 5 h post 60mL MC concentrate or placebo. Significantly different from the placebo: * $p < 0.05$, ** $p < 0.01$.