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1           **Title: Polyphenol-rich tart cherries (*Prunus Cerasus*, cv**  
2           **Montmorency) improve sustained attention, feelings of alertness**  
3           **and mental fatigue and influence the plasma metabolome in middle-**  
4           **aged adults: a randomised, placebo-controlled trial**

5           **Running Title: Cherries and cognitive function**

6  
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## Abstract

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There is accumulating evidence for the protective effects of polyphenols on risk factors associated with cognitive decline and mood disorders. Tart Montmorency cherries (MC) are a particularly rich source of anthocyanins and other polyphenols that have been shown to elicit antioxidant, anti-inflammatory and vasomodulatory actions. This study aimed to determine the influence of chronic MC supplementation on cognitive function, mood, sleep, health and cerebral blood flow. In a 3-month double-blinded, placebo-controlled parallel study, middle-aged adults (mean  $\pm$  SD: 48  $\pm$  6 years) were randomly assigned to either 30 ml twice daily of MC (n = 25) or the same amount of an isocaloric placebo (n = 25). Cognitive function and mood were assessed before and after supplementation using a computerised cognitive task battery and visual analogue scales. Cerebral blood flow was also monitored by near-infrared spectroscopy during the task battery, questionnaires were administered to determine subjective sleep and health status and plasma metabolomics was analysed before and after supplementation. After 3 months, the MC resulted in higher accuracy in digit vigilance (mean difference: 3.3, 95%CI: 0.2, 6.4%) with lower number of false alarms (mean difference: -1.2, 95%CI: -2.0, -0.4) compared to the placebo. There was also a treatment effect for higher alertness (mean difference: 5.9, 95%CI: 1.3, 10.5%) and lower mental fatigue ratings (mean difference -9.5, 95%CI: -16.5, -2.5%) with MC. Plasma metabolomics revealed an increase in a number of amino acids in response to MC intake, but not placebo. These data suggest an anti-fatiguing effect of MC supplementation as well as the ability to improve sustained attention during times of high cognitive demand, this might be related to changes in amino acid metabolism.

**Key words:** anthocyanins; cerebral blood flow, sleep, sustained attention, mental fatigue, Bond-Lader, metabolomics

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## Introduction

65 Cognitive decline is the deterioration of cognition that typically occurs with age.  
66 Moreover, progressive cognitive decline is implicated in the pathophysiology of  
67 neurodegenerative diseases and mood disorders <sup>(1; 2)</sup>. Deteriorations in cognitive  
68 function happen gradually, commencing in early adulthood and progressing more  
69 rapidly during mid-life <sup>(3; 4)</sup>. Reduced cognitive function is among the most feared  
70 aspects of growing older and in the UK and cognitive failure is the cause for 40% of  
71 admissions to institutional care <sup>(5)</sup>. Hence, maintaining good cognitive function and  
72 mental health is important in healthy ageing <sup>(6)</sup>. Given the global ageing population and  
73 the inherent economic, personal and societal burdens related to poor cognition,  
74 delaying cognitive ageing, reducing the disease risk trajectory and preventing  
75 neurodegenerative diseases has become a research priority. Neurodegenerative  
76 diseases and mood disorders have some commonality in the underpinning  
77 mechanisms that might be related to increased exposure and impaired ability for  
78 defence mechanisms to resist oxidative stress and inflammation as well as impaired  
79 vascular function and cerebral blood flow (CBF) <sup>(7; 8; 9)</sup>. Thus, dietary sources of  
80 polyphenols, that have been shown to improve these factors, which might serve to  
81 maintain better cognitive function have become a topic of interest <sup>(10)</sup>. For example, in  
82 a recent longitudinal study of middle-aged adults from the Framingham Offspring  
83 Cohort, highest compared to the lowest dietary anthocyanin intake was associated  
84 with lower risk of developing Alzheimer's disease and related dementia over a 19.7  
85 year follow-up <sup>(11)</sup>.

86 Anthocyanins (from the Greek *anthos*, a flower, and *kyanos*, dark blue) are a subclass  
87 of polyphenols responsible for the red and blue pigmentation in fruits and vegetables

88 (12). Tart Montmorency cherries (MC) are a rich source of anthocyanins and other  
89 phytochemicals [e.g. (poly)phenols, carotenoids and indolamines (13)] that have been  
90 demonstrated to cross the blood-brain-barrier (14; 15). Tart MC phytochemicals have  
91 also been reported to exert anti-neuro-inflammatory properties and to suppress  
92 neuronal apoptosis and stimulate pro-survival signalling cascades - mechanisms that  
93 might protect against cognitive ageing (16; 17; 18). Additionally, anthocyanins have also  
94 been shown to upregulate brain derived neurotrophic factor (BDNF), a potential  
95 mechanism and plausible link between dietary anthocyanin intake and improved  
96 cognition, particularly memory (19; 20). In accordance, Thangthaeng and colleagues (18)  
97 reported improvements in working memory, markers of inflammation and autophagy  
98 in aged Fischer rats following 6-week supplementation with MC powder compared to  
99 a control. Other possible benefits include the potential for MC anthocyanins to  
100 enhance blood flow that could result in improved delivery and uptake of oxygen and  
101 glucose to the brain to support optimal cerebral functioning (21; 22; 23). Moreover,  
102 increased endothelial dysfunction, inflammation and poor sleep are closely associated  
103 to depression (24; 25); and MC have been shown to have favourable influences on these  
104 (26; 27) suggesting a putative role in cognitive function and mood.

105 However, evidence from human trials regarding the influence of cherries on mood and  
106 cognition are less consistent. For example, acute cherry intake has not been shown  
107 to influence cognitive performance, despite modulating blood flow (21; 28). Nevertheless,  
108 longer-term cherry supplementation has been shown to improve some aspects of  
109 cognitive performance (29; 30). Moreover, both aforementioned studies (29; 31) were  
110 predicated by reductions in systolic blood pressure, suggesting that the vasodilatory  
111 properties of the cherries might be at least partly driving this response. Despite these

112 findings, at present, no attempt has been made to examine the cerebral  
113 haemodynamic response to chronic tart cherry supplementation in response to  
114 cognitive tasks. Furthermore, as the only longer-term cherry studies have been in older  
115 adults it is not known whether these findings extend to other populations, and certainly  
116 there is evidence that midlife might be a critical period to intervene <sup>(32; 33)</sup>. It was  
117 therefore hypothesised that MC would improve cognitive function and mood and  
118 increase cerebral blood flow. In this context, as part of a larger study, we aimed to  
119 determine the influence of 3-month supplementation with MC on cognitive function,  
120 mood, sleep, health and cerebral blood flow in middle-aged adults.

## 121 **Methods**

### 122 *Participants*

123 Fifty non-smoking adults (34/16 males/females; mean  $\pm$  SD age:  $48 \pm 6$  years and  
124 BMI:  $27.6 \pm 3.7$  kg/m<sup>2</sup>) out of 56 recruited completed the present randomised, double-  
125 blind, placebo-controlled, parallel-arm study (Figure 1). The participant inclusion and  
126 exclusion criteria has been previously reported <sup>(34)</sup>; briefly, to be included in the study,  
127 participants had low intake of fruit and vegetable (<5 servings/day) and levels of  
128 physical activity ( $\leq 4$  hours/week of moderate-vigorous activity) and  $\geq 1$  additional risk  
129 factor for type II diabetes <sup>(35; 36)</sup>. The study was conducted in accordance with the  
130 Declaration of Helsinki and ratified by the University's Research Ethics Committee  
131 prior to participants providing written, informed consent. This study was part of a larger  
132 trial examining other health indices associated with polyphenol intake that was  
133 registered as a clinical trial (clinicaltrials.gov; NCT04021342); with *a priori* power  
134 calculation based on systolic blood pressure the primary outcome. A *post hoc* power

135 analysis was calculated using G\*Power (version 3.1.9.6, Germany) based on the effect  
136 size of the significant findings which suggested sufficient power ( $1-\beta = 1.00$ ;  $\alpha = 0.05$ ;  
137  $n = 50$ ) for the current study.

### 138 *Procedures*

139 Each participant was required to attend the laboratory on three separate occasions.  
140 On the first visit, participants were screened for inclusion/exclusion criteria. If deemed  
141 eligible, they were familiarised with the cognitive function tasks using voice recorded  
142 instructions <sup>(37)</sup>, following which they were then randomly assigned using computer  
143 generated plan (randomization.com) 1:1; stratified by sex, to receive either 30 ml,  
144 twice daily of MC concentrate ( $n = 25$ ) or an isocaloric placebo ( $n = 25$ ) for 3 months.  
145 We have previously shown that 60 ml of MC is physiologically relevant and well  
146 tolerated <sup>(21; 22)</sup> and other studies had found benefits after bi-daily supplementation  
147 strategies <sup>(31; 38)</sup>. The sleep, cognitive function, mood, health and cerebral blood flow  
148 outcomes were assessed over two experimental visits, (visit 2; pre-supplementation)  
149 and at 3 months (visit 3; post-supplementation). Visit 2 was preceded by a minimum  
150 of a 7-day low anthocyanin run-in in which berry fruits, red grapes (including extracts/  
151 juices) and red wine <sup>(39; 40)</sup> to  $\leq 1$  portion per day. Both experimental visits took place at  
152 9:00 am  $\pm 1$  h and were preceded by an overnight fast ( $\geq 10$ h). Participants were also  
153 asked to arrive hydrated and to avoid strenuous exercise, alcohol, nutritional  
154 supplements for 24 hours and caffeine 12 for hours prior. Throughout the study  
155 participants were encouraged to maintain their habitual diet and exercise routines,  
156 however they were asked to refrain from consuming cherries, cherry products, or any  
157 antioxidant supplements and to limit the aforementioned anthocyanin rich foods to one  
158 or less portion per day throughout the study period. Participants recorded their pre-

159 evening meal before experimental visit one and were asked to replicate this before the  
160 second experimental visit. Participants completed an estimated 3-day diet diary (two  
161 consecutive weekdays and one weekend day), before and the International Physical  
162 Activity Questionnaire (IPAQ; <sup>(41)</sup>) on the day of each experimental visit. Analysis of  
163 food diaries and IPAQ indicated 100% adherence to dietary restrictions and no  
164 changes in energy intake or physical activity over the study duration (Supplementary  
165 Table 1). Participants total polyphenol (flavonoids, phenolic acids, stilbenes, and  
166 lignans) and anthocyanin intake was estimated from their 3-day diet diary using  
167 Phenol-explorer <sup>(42)</sup> and is presented in Table 2. Anthocyanin intake was not different  
168 between groups but on average the mean intake of total polyphenols was ~244 mg/day  
169 higher in the cherry group, with the highest polyphenol contribution was from coffee  
170 (56%) and tea (26%).

### 171 *Treatments*

172 The MC concentrate was supplied by Cherry Marketing Institute (USA) which was  
173 stored at 4°C as directed. Two different batches of the MC concentrate were examined  
174 for total anthocyanins and total phenolic content using techniques previously  
175 described <sup>(22)</sup> and found to contain on average 370.2 (SD: 112.2) mg/L of cyanidin-3-  
176 glucoside equivalents and 3259.0 (SD: 218.9) mg/L gallic acid equivalents,  
177 respectively. The placebo supplement consisted of unsweetened black cherry  
178 flavoured Kool-Aid (Kraft Foods Ltd.), dextrose (MyProtein Ltd.), fructose (Sports  
179 Supplements Ltd.), lemon juice, artificial food colouring (E129 and E133) and bottled  
180 water <sup>(38; 43)</sup>. Both drinks were isocaloric (Table 1) and were packaged in the same  
181 polyethylene terephthalate containers, hence similar visual properties. Participants  
182 were instructed to dilute each 30 ml serving in 240 ml of water as recommended. To

183 ensure blinding participants were given their assigned treatment by a researcher  
184 independent to the project along with a 30 ml measuring, blinding was also assessed  
185 by treatment guess on the last experimental visit. Treatment compliance was  
186 measured by daily tick sheets and return of any unconsumed juice.

### 187 *Cerebral blood flow*

188 Changes in cerebral blood flow; CBF was assessed using continuous wave near infra-  
189 red spectroscopy; NIRS (NIRO-200NX, Hamamatsu Photonics K.K., Japan). Two  
190 near-infrared sensors were placed over the left and right frontal lobe region of the  
191 forehead corresponding to the International 10–20 system Fp1 and Fp2 EEG  
192 positions; these signals were averaged to determine cerebral oxygenation. The  
193 sensors were secured to the skin using double-sided adhesive tape and shielded from  
194 ambient light using an elastic head band. The emitter/optode separation distance of 4  
195 cm. A 5-minute rest (which acted as the NIRS baseline for CBF calculations) was  
196 taken at each testing session and data were acquired continuously throughout a  
197 cognitive task battery. Output was time-stamped at each task segment and averaged  
198 over the task period. Baseline adjusted data with respect to the 5 min of NIRS data  
199 collected immediately prior to completing the tasks <sup>(44;45)</sup>, was calculated offline. NIRS  
200 data are reported as changes in cerebral oxy- (HbO<sub>2</sub>), deoxy- (hHb) and total-(tHb)  
201 haemoglobin concentrations.

### 202 *Cognitive function, mood, sleep and health assessment*

203 Participants completed the Pittsburgh Sleep Quality Inventory, (PSQI; <sup>(46)</sup>) and a short  
204 form quality-of-life survey (SF-36; <sup>(47)</sup>) to assess sleep quality and health, respectively.  
205 The PSQI is a subjective measure of the quality and pattern of sleep over the past 30

206 days. Questions relate to seven domains (subjective sleep quality, sleep latency, sleep  
207 efficiency, sleep duration, sleep disturbances, daytime dysfunction, and use of  
208 medication to assist sleep) and a global score is given, with a higher score indicating  
209 “poorer sleep”. The SF-36 was used to assess personal perception of general health  
210 (average of 5 components) before and after the intervention. The single item of health  
211 changes in the last year was also included to determine any major self-reported  
212 changes in health status. Cognitive function and mood measures were assessed using  
213 a test battery administered via the Computerised Mental Performance Assessment  
214 System (COMPASS, Northumbria University, Newcastle upon Tyne, UK), a purpose-  
215 designed software application for the flexible delivery of randomly generated parallel  
216 versions of standard and novel cognitive assessment tasks <sup>(22; 45)</sup>. The test battery  
217 included three tasks; digit vigilance (DV; 3 min), rapid visual information processing  
218 (RVIP; 5 min) and *N*-back task (~3 min). The cognitive tests (described below) were  
219 repeated twice in order to induce cognitive fatigue, which was assessed immediately  
220 after each battery by a visual analogue scale (VAS). The VAS was presented as  
221 ‘mental fatigue’ in which participants had to mark on a line scale anchored “not at all”  
222 (left hand end) and “very much so” (right hand end); with higher scores representing  
223 more mental fatigue. Participants also completed Bond-Lader VAS <sup>(48)</sup> before and after  
224 the cognitive function tests to assess subjective mood.

### 225 *Bond-Lader VAS*

226 The VAS required participants to indicate how they currently feel “at this moment in  
227 time” by clicking, using the mouse, at the appropriate point along a 100 mm scale on  
228 screen. Sixteen scales are presented with antonyms at either end, e.g. ‘alert’ vs.  
229 ‘drowsy’, ‘lethargic’ vs. ‘energetic’ and ‘troubled’ vs. ‘tranquil’, with these 16 scores (%)

230 along the line towards the right end) combining to create three overall measures of  
231 mood factors: 'alert', 'content' and 'calm'.

### 232 *Digit Vigilance (DV)*

233 The DV task is a measure of sustained attention and psychomotor speed. A single  
234 target digit was randomly selected and constantly displayed on the right-hand side of  
235 the screen. A series of single digits appeared on the left-hand side of the screen, one  
236 at a time, at the rate of 150 per minute. The participant was required to press the  
237 spacebar on the keyboard as quickly as possible every time the digit in the series  
238 matched the target digit. Task outcomes included accuracy (%) and reaction time for  
239 correct responses (ms) and number of false alarms. This task has been shown to  
240 identify age-related declines in attention and the test-retest correlation coefficient for  
241 reaction time is 0.81 <sup>(49)</sup>.

### 242 *Rapid Visual Information Processing (RVIP)*

243 The RVIP task is a measure of sustained attention and working memory. The task  
244 requires the participant to monitor a continuous series of single digits for targets of  
245 three consecutive odd or three consecutive even digits. The digits are presented on  
246 the computer screen one at a time at the rate of 100 per minute in pseudo-random  
247 order, and the participant responds to the detection of a target string by pressing the  
248 spacebar on the keyboard as quickly as possible. Task outcomes included number of  
249 target strings correctly detected (%) and average reaction time for correct detections  
250 (ms) and number of false alarms. The test-retest correlation for these is (>0.70) in  
251 older adults and has been reported as reliable in the detection and monitoring of  
252 cognitive deficits <sup>(50)</sup>.

253 *N-Back*

254 The 3-back task measures working memory and memory capacity. The task requires  
255 participants to indicate whether the letter presented on screen was also presented 3  
256 letters previously in the letter sequence. Participants are required to respond by  
257 pressing buttons corresponding to 'yes' or 'no' on the keyboard, to each letter, as  
258 quickly as they can. Participants were presented with 45 stimuli (letters); however, the  
259 task is dependent on speed (i.e. slower reaction times will result in a lengthier task).  
260 The task outcomes included accuracy of correct yes responses (%) and reaction time  
261 for correct yes responses (ms). The test-retest correlation coefficient for this task has  
262 been reported to be 0.73 for accuracy and 0.81 for reaction time, respectively <sup>(51)</sup>.

263 *Metabolomics protocol*

264 Non-targeted metabolomics was performed on plasma samples. Fasted venous blood  
265 samples were collected in lithium-heparin vacutainers (Becton, Dickinson and  
266 Company, USA). Due to blood sampling error samples were only available for 38  
267 participants (n=19 for MC and placebo group) for both time points, baseline and 3  
268 months. These were centrifuged at 3000× g (4°C) for 10 min and the plasma aliquoted  
269 and stored at –80°C.

270 The plasma samples were defrosted on ice and extracted using a biphasic Folch  
271 extraction methodology, as follows: 100 uL of plasma samples were extracted in 300  
272 uL of 2:1 chloroform/methanol solution. The samples were vortex for 1 min and then  
273 allowed to incubated on ice for 30 mins. Next 50 uL of optima grade LC/MS water were  
274 added to solution induced phase separation and vortex for 30 s, the samples were  
275 incubated on ice for additional 10 mins. The extraction buffer was then centrifuged at

276 3000 rpm at 4°C for 15 mins, 100 uL of the aqueous layer collected and filtered via  
277 0.22 micron cellulose filter and transferred to 1.5 autosampler vials with 200 uL  
278 microinsert. Quality controls (QC) samples were also made by pooling 10 uL of each  
279 sample together.

280 Hydrophilic Liquid Interaction Chromatography (HILIC) metabolite profiling of the  
281 plasma samples was performed on a Thermo Scientific (Hemel Hempstead, United  
282 Kingdom) Vanquish Liquid chromatography chromatographic separation system  
283 connected to IDX High Resolution Mass Spectrometer. The HILIC positive and  
284 negative data sets were processed via Compound Discoverer 3.2 according to the  
285 following settings: Untargeted Metabolomic workflow: mass tolerance 10 ppm,  
286 maximum shift 0.3 min, alignment model adaptive curve, minimum intensity  $1^{e6}$ , S/N  
287 threshold 3, compound consolidation, mass tolerance 10 ppm, retention time tolerance  
288 0.3 min. Database matching was performed using Thermo scientific m/z cloud  
289 databased with a similar index of 70% or better MS2 spectra. Those metabolites that  
290 could be matched (n=174) and had a relative standard deviation of 30% or less within  
291 the QCs were retained for analysis.

292 The dataset was autoscaled and cube root transformed using Metaboanalyst 5.0  
293 software <sup>(52)</sup> before performing detailed multivariate and univariate analysis including  
294 Principal Component Analysis (PCA) that was used for identification of outliers. Partial  
295 Least Squares Discriminant Analysis (PLSDA) was used to test for discrimination  
296 between sample MC group at baseline and 3 months. The relative metabolite  
297 abundance of the metabolites from the MC with Variable Importance in Projection  
298 (VIP) factor >1 was then compared to placebo. The PCA identified 2 outlier samples  
299 (Supplemental Figure 1), which were removed before analysis.

### 300 *Statistical analysis*

301 All data were analysed using IBM SPSS statistics (v 26.0 for Windows; SPSS,  
302 Chicago, USA), measures are reported as means  $\pm$  standard deviation (SD) in tables  
303 and standard error (SE) in figures unless otherwise stated. Baseline characteristics  
304 were compared by Wilcoxon signed-rank test where data were continuous and  
305 treatment guess analysed by Chi-square test. Outcome data was cleaned by  
306 generating box plots for each outcome variable to identify potential outliers. Values  
307 that were more than one and a half and three deviations from the interquartile range  
308 were identified as outliers, and extreme outliers, respectively <sup>(53)</sup>, which were removed.  
309 Despite familiarisation with the cognitive function tasks some participants did not  
310 perform the tasks correctly (e.g., by pressing the wrong button), therefore these were  
311 removed before data cleaning. The number of participants analysed for each variable  
312 can be found in the corresponding tables and figures.

313 Health (SF-36) and sleep (PSQI) data were analysed using the MIXED procedure in  
314 SPSS with treatment (cherry juice/placebo) and visit (pre, post) as fixed factors and  
315 participant number as a random factor. The post-dose cognitive and mood outcome  
316 measures were modelled using the MIXED procedure in SPSS which included the  
317 respective baseline values as a covariate and the terms treatment (cherry  
318 juice/placebo) and repetition (1, 2) as fixed factors and participant number as a random  
319 factor.

320 The NIRS data was separated into epochs for each task adjusted for resting baseline  
321 data (5 mins prior). The task length was fixed for the DV (180 s) and RVIP (300 s), but  
322 NIRS data from the N-Back test was truncated so that the same amount of data was

323 analysed for all participants. The epochs were averaged across the 2 channel  
324 hemispheres. If the participant's data had been omitted from the cognitive function  
325 task (for all variable i.e. accuracy, reaction time and false alarms) the epoch was  
326 excluded from analysis for that task. The resting pre-task-adjusted post-dose NIRS  
327 outcome measures were modelled using the MIXED procedure in SPSS which  
328 included the respective baseline pre-task-adjusted values as a covariate and the terms  
329 treatment (cherry juice/placebo) and task epochs (1-6) as fixed factors and participant  
330 number as a random factor. Sidak adjusted *post-hoc* comparisons were then carried  
331 out between cherry juice and placebo as appropriate.

## 332 **Results**

333 The baseline demographics of the cohort were similar regarding age, height, weight  
334 and BMI, ( $P > 0.05$ ). A full list of demographics including education, left-handed and  
335 medication use can be found in Table 2. The study was successfully blinded ( $P =$   
336  $0.386$ ) and the mean ( $\pm$  SD) self-reported treatment compliance was  $94 \pm 15$  %.

### 337 *The effect of MC on cerebral blood flow*

338 After 3-month supplementation there was no treatment or treatment  $\times$  epoch  
339 interaction effects for HbO<sub>2</sub>, hHb or tHb concentrations assessed by NIRS during any  
340 of the tasks (Supplemental Figure 2).

### 341 *The effect of MC on sleep and health*

342 Overall sleep duration across both visits was higher in the MC group (mean difference:  
343  $24.2$ , 95%CI:  $4.8$ ,  $43.6$  mins:  $F = 6.15$ ,  $P = 0.015$ ), main effect of treatment. After 3  
344 months sleep duration had decreased in the MC group  $13.8$  mins and increased in the  
345 placebo group  $11.6$  mins, but there was no interaction ( $F=1.69$ ,  $P = 0.197$ ). There were

346 no differences between treatments after 3 months for subjective sleep assessed by  
347 the PSQI or general health and health change assessed by SF-36 (Table 3).

#### 348 *The effect of MC on cognitive performance and mood*

349 Across repetitions post-supplementation DV accuracy was higher (mean difference:  
350 3.3, 95%CI: 0.2, 6.4%:  $F = 4.57$ ,  $P = 0.035$ ; Figure 2A) and number of false alarms  
351 was lower (mean difference: -1.2, 95%CI: -2.0, -0.4:  $F = 8.49$ ,  $P = 0.005$ ; Figure 2B)  
352 when adjusted for baseline with MC compared to the placebo. There was no treatment  
353 or interaction effects between treatments for any other cognitive function variables  
354 (Table 4).

355 After 3 months the alert Bond-Lader was higher in the MC (mean difference: 5.9,  
356 95%CI: 1.3, 10.5%:  $F = 6.42$ ,  $P = 0.013$ ; Figure 3A), main effect of treatment. Similarly,  
357 post-supplementation mental fatigue VAS was significantly lower (mean difference -  
358 9.5, 95%CI: -16.5, -2.5%; Figure 3B) in the MC group ( $F = 7.21$ ,  $P = 0.009$ ). There was  
359 no effect of the treatment on calm or content Bond-Lader (Table 5).

#### 360 *The effect of MC on plasma metabolome*

361 The PLSDA for all treatments and MC only at baseline and 3 months are presented in  
362 Figure 4, demonstrating a change in plasma metabolome after supplementation with  
363 MC. In total 35 database matched metabolites were shown to be different after 3-  
364 month supplementation with MC ( $VIP > 1$ ; Supplemental Figure 3). Polyphenol  
365 metabolites, quinic acid and 3,4-Dihydroxybenzenesulfonic acid as well as several  
366 amino acids; 3-methylhistidine, L-phenylalanine, betaine, L-serine, choline  
367 upregulated after post-supplementation with MC but not placebo, Figure 5.

368

## Discussion

369 The main finding of this study was that tart cherries have a positive impact on cognitive  
370 performance and perceptions of fatigue and alertness and upregulate plasma amino  
371 acids, with no influence on CBF, sleep or health. In the current study MC improved  
372 sustained attention measured by DV. Both sweet <sup>(29)</sup> and tart <sup>(31)</sup> cherries have been  
373 shown to improve aspects of cognitive function following 12-week supplementation in  
374 older adults, including sustained attention, however it is currently unknown whether  
375 this is a result of improved CBF or due to the potential neuroprotective properties of  
376 tart cherry anthocyanins <sup>(17)</sup>. Therefore, we measured blood flow with NIRS placed  
377 over the prefrontal cortex, but no changes in cognitive function or CBF in response to  
378 MC intake were observed. There is no directly comparable study, and hence this  
379 represents the first study to determine cognitive performance and NIRS in response  
380 to chronic supplementation of MC. Although our research group has shown that an  
381 acute bolus of MC can influence CBF <sup>(22)</sup>, we did not observe any influence following  
382 chronic supplementation in the current study. This is likely to be attributable to the  
383 vasomodulatory properties of the cherries coincide with peak plasma concentrations  
384 of anthocyanin metabolites, which are rapidly metabolised and/or excreted <sup>(21)</sup>. It is  
385 therefore conceivable that changes in vascular function are relatively transient with the  
386 bioavailability of the phytonutrients and hence any possible changes from the previous  
387 day had passed. This is consistent with our finding that 3 month supplementation had  
388 no influence on vascular function variables after an overnight fast <sup>(34)</sup>. Moreover, the  
389 data in the present study are in line with previous studies that reported that both  
390 resveratrol <sup>(45)</sup> and *Sideritis scardica* <sup>(54)</sup> supplementation induced acute, but not  
391 chronic changes in CBF parameters measured by NIRS. Furthermore, in a recent  
392 review of the influence of polyphenols on CBF, changes following longer-term

393 supplementation were only apparent in studies using magnetic resonance imaging  
394 (MRI), highlighting the difficulty in detecting changes in CBF <sup>(55)</sup>. For instance, Bowtell  
395 *et al.* <sup>(56)</sup> reported regional changes in brain perfusion measured by MRI and improved  
396 cognitive performance following 12-week supplementation with anthocyanin-rich  
397 blueberry concentrate. In the current study, we used continuous wave NIRS and the  
398 limitations surrounding this are well documented <sup>(57; 58)</sup>, namely it only measure relative  
399 changes in cerebral activation and CBF as opposed to the measurement of absolute,  
400 quantifiable, amounts of haemoglobin present within the cortex. Furthermore, NIRS  
401 was measured on the prefrontal cortex and is not representative of changes elsewhere  
402 within the cerebral cortex and subsequently could be an area for future research to  
403 explore.

404 In the current study MC supplementation resulted in lower self-reported mental fatigue  
405 and higher alertness. Since these effects were mirrored with increased accuracy and  
406 reduced false alarms in the DV task, it would appear that the anti-fatiguing effects of  
407 MC could potentially enhance attention and protect against errors. Moreover, this  
408 sustained attention could be beneficial in various daily tasks, such as driving and  
409 working <sup>(59)</sup>. Only one other study has examined the influence of MC on these aspects  
410 of mood, in which an acute bolus of MC had no effect <sup>(22)</sup>, even though CBF was  
411 modulated suggesting this might not be the driving mechanism. Other studies have  
412 suggested that polyphenol-rich foods such as cocoa, might influence mood after  
413 chronic, but not acute intake <sup>(60; 61)</sup>, albeit the potential underlying mechanisms are yet  
414 to be elucidated. As part of an exploratory analysis for mechanistic understanding we  
415 analysed the plasma metabolome of the participants before and after  
416 supplementation. Three month supplementation of MC resulted in the upregulation of

417 some phenolic acid metabolites (e.g. quinic acid <sup>(62)</sup> and 3,4-  
418 Dihydroxybenzenesulfonic acid) which was not apparent in the placebo. Interestingly,  
419 we also found that MC supplementation upregulated phenylalanine (a precursor to  
420 tyrosine) and histidine metabolism in line with a previous small pilot study <sup>(63)</sup>. These  
421 amino acids have also been shown to be modulated after short-term intake of red wine  
422 and grape polyphenols, which the authors speculate might be due to polyphenols  
423 effecting colonic protein fermentation or changing microbial amino acid metabolism <sup>(64)</sup>  
424 that might be related to prebiotic actions. There was also upregulation of choline,  
425 betaine and serine, which might represent modulation of cholinergic metabolism and  
426 is important for attention and cognition <sup>(65; 66; 67)</sup> and histidine supplementation has  
427 been shown to improve feelings of mental fatigue <sup>(68)</sup>. The ability for MC to modulate  
428 amino acids related to neurotransmitters and cognitive function in the current study  
429 has limited comparability to other studies, but importantly is supported by previous  
430 data <sup>(69)</sup>, and warrants further investigation to understand potential mechanisms of  
431 action.

432 There were no differences found in sleep measures between groups after the  
433 intervention, as assessed by the PSQI. This contradicts previous research which  
434 showed that tart cherries, due to their melatonin content, improved sleep quality <sup>(27; 43;</sup>  
435 <sup>70)</sup>. However, this is likely because of the use of a questionnaire in this study rather  
436 than any objective measures of sleep quality. For example, previous research reported  
437 improved sleep efficiency and total sleep time measured by actigraphy, following 7-  
438 day consumption of MC, but the same measures collected by questionnaires did not  
439 differ <sup>(27)</sup>. Importantly there were no substantial changes in the sleep patterns of  
440 participants, but future studies might employ more quantitative markers. Similarly,

441 other subjective, but nonetheless validated measures like the Bond-Lader and mental  
442 fatigue VAS might need to be considered in a similar light.

443 Other limitations within the current study include that baseline polyphenol intake was  
444 different between groups. Secondly, as discussed elsewhere <sup>(34)</sup>, the sugar content of  
445 MC and potential variability between batches need to be carefully considered in future  
446 research designs <sup>(71)</sup>. Thirdly, based on emerging evidence it is speculated that  
447 (poly)phenols might be beneficial compounds, however it should be acknowledged  
448 that MC contains other phytochemicals that might synergistically have an effect <sup>(30; 72;</sup>  
449 <sup>73)</sup>. Lastly, metabolomics was only conducted on compounds that could be matched to  
450 the database, but it should be acknowledged that other important compounds could  
451 have been missed. Notwithstanding, due to the low number of adverse events, good  
452 compliance levels reported and no effect on subjective health as assessed by the SF-  
453 36, it is reasonable to suggest that 60 ml/day of MC is a safe and tolerable intervention.  
454 Moreover, to date this is the first study to determine the effect of chronic  
455 supplementation of MC on cognitive function in middle-aged adults. To the best of our  
456 knowledge this is also the only study in tart cherries to concurrently examine the CBF  
457 mechanism and plasma metabolome to cognitive outcomes following longer-term  
458 supplementation. Therefore, this study provides insight into the effects of tart cherries  
459 on cognitive performance and mood in middle-aged adults, which might be related to  
460 the ability to modulate amino acid metabolism and provides a platform for future  
461 research.

462 In conclusion, the current study reports higher sustained attention, self-reported  
463 alertness and lower mental fatigue following supplementation with MC. The  
464 intervention also appeared to upregulate amino acids that might indicate a potential

465 underlying mechanism. These data provide new information that bioactive foods that  
466 are rich in anthocyanins and other (poly)phenolic compounds, can have an anti-  
467 fatiguing effect during periods of high cognitive demand, which are beneficial in daily  
468 tasks requiring vigilance.

469 **Author Contributions:** RK, KMK, JKL, CH and GH: conceived and designed the  
470 research; RK: conducted the research; RK, CH and GH analysed and interpreted the  
471 data; RK and GH: drafted the manuscript and had primary responsibility for the final  
472 content; and all authors: read and approved the final manuscript. The authors declare  
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- 683

**Table 1.** Nutritional composition of treatments per 60 ml 684

	<b>MC concentrate</b>	<b>Placebo</b>
Energy (kcal)	204	204
Carbohydrate (g)	50	51
Protein (g)	2.2	0.0
Fat (g)	0.0	0.0
TACN (mg) mean $\pm$ SD <sup>a</sup>	22.2 $\pm$ 6.7	-
TPC (mg) mean $\pm$ SD <sup>b</sup>	195.5 $\pm$ 13.1	21.5 $\pm$ 2.3

TACN; total anthocyanin content (cyanidin 3 glucoside equivalents); TPC; total polyphenol content (gallic acid equivalents).

<sup>a</sup>Analysed by pH-differential method (placebo was not analysed because it contained artificial colourant (E129) which causes interference with the assay <sup>(74)</sup>).

<sup>b</sup>Analysed using a modified Folin-Ciocalteu colorimetric method.

**Table 2.** Baseline characteristics of participants

<b>Variable</b>	<b>Cherry (n = 25)</b>	<b>Placebo (n = 25)</b>	<b>P-Value</b>
<b>Age (y)</b>	49 ± 6	47 ± 6	0.465
<b>Sex (m/f)</b>	8/17	8/17	
<b>Stature (cm)</b>	173.8 ± 9.2	173.2 ± 8.9	0.884
<b>Body Mass (kg)</b>	82.9 ± 13.9	82.9 ± 12.5	0.968
<b>BMI (kg/m<sup>2</sup>)</b>	27.3 ± 3.8	27.5 ± 3.8	0.570
<b>Anthocyanins (mg/day)</b>	9.7 ± 17.2	17.8 ± 40.5	0.958
<b>Total polyphenols (mg/day)</b>	571.8 ± 244.9	327.9 ± 195.8	0.010
<b>Education (n; %)</b>			
Less than high school	-	-	
High school or equivalent	9 (36)	12 (48)	
Bachelor's degree	9 (36)	9 (36)	
Postgraduate degree	7 (28)	4 (16)	
<b>Left-handed (n; %)</b>	3 (12)	1 (4)	
<b>Regular use of medication (n; %)*</b>	6 (24)	6 (24)	
Blood pressure	2 (8)	2(8)	
Cholesterol	1 (4)	2(8)	
HRT	1 (4)	-	
Antidepressant	-	2(8)	
Gout	1 (4)	-	
ADHD	-	1(4)	
Asthma	1 (4)	-	

685

Data is presented as mean ± SD unless otherwise stated.

\*Medication stabilised for ≥ 3 months

Attention deficit hyperactivity disorder (ADHD); hormone replacement therapy (HRT)

**Table 3.** Subjective sleep quality assessed by Pittsburgh Sleep Quality Inventory (PSQI) and health assessed by Short form-36 before and after supplementation with tart Montmorency cherry concentrate or an isocaloric placebo.

	n	Cherry	Placebo	Mixed Model	
				Effect	P-Value
<b>Sleep Latency (mins)</b>	49				
Baseline		16.3 ± 9.4	17.6 ± 9.6	T	0.672
3 months		20.8 ± 14.6	21.7 ± 13.5	T*V	0.932
<b>Sleep Duration (mins)*</b>	50				
Baseline		434 ± 63	398 ± 28	T	<b>0.015</b>
3 months		421 ± 54	409 ± 38	T*V	0.197
<b>Habitual Sleep Efficiency (%)</b>	50				
Baseline		90.0 ± 7.0	86.8 ± 7.1	T	0.308
3 months		84.7 ± 8.5	84.5 ± 10.9	T*V	0.380
<b>Global PSQI score</b>	49				
Baseline		4.0 ± 1.6	5.0 ± 1.8	T	0.092
3 months		4.3 ± 1.6	4.5 ± 1.9	T*V	0.278
<b>General health (%)</b>	49				
Baseline		67 ± 19	71 ± 18	T	0.462
3 months		68 ± 13	69 ± 15	T*V	0.569
<b>Health change (%)</b>	47				
Baseline		58 ± 12	58 ± 14	T	0.269
3 months		56 ± 11	51 ± 14	T*V	0.391

Data is presented as mean ± SD

Effects are treatment (T) and treatment by visit interaction (T\*V). \*Significant difference between treatments (P < 0.05).

**Table 4.** Cognitive function tasks before and after supplementation with tart Montmorency cherries or an isocaloric placebo (data are presented as Mean  $\pm$  SD)

Measure	Treatment	n	Baseline		3 months		Mixed Model	
			Rep 1	Rep 2	Rep 1	Rep 2	Effect	P-Value
<b>DV accuracy (%)*</b>	Cherry	45	94.1 $\pm$ 5.9	88.2 $\pm$ 13.1	95.7 $\pm$ 3.5	88.6 $\pm$ 11.6	T	<b>0.035</b>
	Placebo		93.0 $\pm$ 6.0	84.7 $\pm$ 14.7	89.0 $\pm$ 9.8	85.9 $\pm$ 10.3	T*R	0.294
<b>DV RT (ms)</b>	Cherry	46	477.8 $\pm$ 37.8	492.5 $\pm$ 39.5	477.4 $\pm$ 33.9	495.3 $\pm$ 38.8	T	0.166
	Placebo		473.5 $\pm$ 31.4	500.1 $\pm$ 37.2	484.7 $\pm$ 37.5	511.4 $\pm$ 34.2	T*R	0.619
<b>DV FA (n)*</b>	Cherry	41	2.5 $\pm$ 1.9	2.5 $\pm$ 2.3	1.4 $\pm$ 1.3	3.1 $\pm$ 2.3	T	<b>0.005</b>
	Placebo		2.5 $\pm$ 1.9	3.8 $\pm$ 1.9	3.6 $\pm$ 2.8	4.5 $\pm$ 2.8	T*R	0.182
<b>RVIP accuracy (%)</b>	Cherry	42	57.2 $\pm$ 17.3	63.0 $\pm$ 18.3	63.0 $\pm$ 20.9	71.5 $\pm$ 15.3	T	0.194
	Placebo		50.4 $\pm$ 18.7	53.8 $\pm$ 17.2	56.3 $\pm$ 21.8	56.7 $\pm$ 20.5	T*R	0.765
<b>RVIP RT (ms)</b>	Cherry	41	555.6 $\pm$ 62.1	568.1 $\pm$ 50.6	555.5 $\pm$ 53.5	549.6 $\pm$ 35.0	T	0.539
	Placebo		557.9 $\pm$ 63.9	540.1 $\pm$ 47.3	536.4 $\pm$ 51.7	545.1 $\pm$ 37.1	T*R	0.351
<b>RVIP FA (n)</b>	Cherry	44	5.9 $\pm$ 3.8	2.8 $\pm$ 1.3	5.5 $\pm$ 5.2	3.8 $\pm$ 2.6	T	0.955
	Placebo		4.9 $\pm$ 3.2	5.2 $\pm$ 3.8	4.3 $\pm$ 3.1	4.7 $\pm$ 2.6	T*R	0.786
<b>3-Back accuracy (%)</b>	Cherry	40	80.2 $\pm$ 5.4	85.3 $\pm$ 8.4	79.7 $\pm$ 9.1	85.4 $\pm$ 9.0	T	0.608
	Placebo		76.9 $\pm$ 9.3	81.8 $\pm$ 8.8	77.5 $\pm$ 9.4	83.3 $\pm$ 7.9	T*R	0.962
<b>3-Back RT (ms)</b>	Cherry	40	1110 $\pm$ 231	980 $\pm$ 234	1111 $\pm$ 248	942 $\pm$ 195	T	0.930
	Placebo		946 $\pm$ 239	901 $\pm$ 205	1054 $\pm$ 286	920 $\pm$ 247	T*R	0.653

Abbreviations; Digit vigilance (DV); false alarm (FA); rapid visual image processing (RVIP); reaction time (RT); repetition (Rep). Effects are treatment (T) and treatment by repetition interaction (T\*R). \* Significant difference between treatments ( $P < 0.05$ ).

**Table 5.** Mood and visual analogue scale measures before and after supplementation with tart Montmorency cherries or an isocaloric placebo (data are presented as Mean  $\pm$  SD)

Measure	Treatment	n	Baseline		3 months		Mixed Model	
			Rep 1	Rep 2	Rep 1	Rep 2	Effect	P-Value
<b>Alert (%)*</b>	Cherry	48	71.8 $\pm$ 9.5	52.6 $\pm$ 14.8	69.3 $\pm$ 11.3	59.7 $\pm$ 18.5	T	<b>0.013</b>
	Placebo		71.8 $\pm$ 11.1	47.8 $\pm$ 14.2	65.6 $\pm$ 15.3	43.2 $\pm$ 14.8	T*R	
<b>Content (%)</b>	Cherry	48	77.5 $\pm$ 9.2	68.1 $\pm$ 12.6	81.0 $\pm$ 7.9	74.4 $\pm$ 12.2	T	0.166
	Placebo		76.8 $\pm$ 10.9	60.5 $\pm$ 18.9	75.8 $\pm$ 13.8	66.1 $\pm$ 17.9	T*R	
<b>Calm (%)</b>	Cherry	49	74.2 $\pm$ 9.6	57.3 $\pm$ 13.5	76.0 $\pm$ 13.1	64.7 $\pm$ 15.3	T	0.699
	Placebo		65.1 $\pm$ 18.0	54.4 $\pm$ 13.1	74.8 $\pm$ 13.7	61.8 $\pm$ 18.4	T*R	
<b>Mental fatigue (%)*</b>	Cherry	46	53.5 $\pm$ 18.1	72.3 $\pm$ 10.5	48.8 $\pm$ 21.9	56.0 $\pm$ 22.9	T	<b>0.009</b>
	Placebo		59.0 $\pm$ 15.1	72.4 $\pm$ 21.7	56.6 $\pm$ 13.3	64.3 $\pm$ 24.6	T*R	

Abbreviations; repetition (rep). Effects are treatment (T) and treatment by repetition interaction (T\*R). \* Significant difference between treatments ( $P < 0.05$ ).

**Figure 1.** Consort diagram of study enrolment, allocation and analysis

**Figure 2.** Estimated marginal means and standard error (SE) for post-treatment digit vigilance (DV) accuracy (A; n = 45) and false alarms (B; n = 41). \*P<0.05 between treatments.

**Figure 3.** Estimated marginal means and standard error (SE) for post-treatment alert Bond-Lader (A; n = 48) and mental fatigue VAS (B; n = 46). \*P<0.05 between treatments.

**Figure 4.** Partial Least Squares Discriminant Analysis (PLS-DA) for all treatments (left) and cherry group only (right).

**Figure 5.** Original and normalised concentration of metabolites upregulated in the cherry but not placebo group post-supplementation.