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

Running Title: Cherries and cognitive function

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Abstract

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 ± SD: 48 ± 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

Introduction

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

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

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

However, evidence from human trials regarding the influence of cherries on mood and cognition are less consistent. For example, acute cherry intake has not been shown to influence cognitive performance, despite modulating blood flow [21; 28]. Nevertheless, longer-term cherry supplementation has been shown to improve some aspects of cognitive performance [29; 30]. Moreover, both aforementioned studies [29; 31] were predicated by reductions in systolic blood pressure, suggesting that the vasodilatory 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 [32; 33]. 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 ± SD age: 48 ± 6 years and
124 BMI: 27.6 ± 3.7 kg/m^2) 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 [34]; 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 (≤4 hours/week of moderate-vigorous activity) and ≥1 additional risk
129 factor for type II diabetes [35; 36]. 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

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

Procedures

Each participant was required to attend the laboratory on three separate occasions. On the first visit, participants were screened for inclusion/exclusion criteria. If deemed eligible, they were familiarised with the cognitive function tasks using voice recorded instructions [37], following which they were then randomly assigned using computer generated plan (randomization.com) 1:1; stratified by sex, to receive either 30 ml, twice daily of MC concentrate (n = 25) or an isocaloric placebo (n = 25) for 3 months. We have previously shown that 60 ml of MC is physiologically relevant and well tolerated [21; 22] and other studies had found benefits after bi-daily supplementation strategies [31; 38]. The sleep, cognitive function, mood, health and cerebral blood flow outcomes were assessed over two experimental visits, (visit 2; pre-supplementation) and at 3 months (visit 3; post-supplementation). Visit 2 was preceded by a minimum of a 7-day low anthocyanin run-in in which berry fruits, red grapes (including extracts/ juices) and red wine [39; 40] to ≤1 portion per day. Both experimental visits took place at 9:00 am ± 1 h and were preceded by an overnight fast (≥ 10h). Participants were also asked to arrive hydrated and to avoid strenuous exercise, alcohol, nutritional supplements for 24 hours and caffeine 12 for hours prior. Throughout the study participants were encouraged to maintain their habitual diet and exercise routines, however they were asked to refrain from consuming cherries, cherry products, or any antioxidant supplements and to limit the aforementioned anthocyanin rich foods to one 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; [41]) 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 [42] 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 [22] 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 [38; 43]. 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

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

Cerebral blood flow

Changes in cerebral blood flow; CBF was assessed using continuous wave near infra-red 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 head band. The emitter/optode separation distance of 4 cm. A 5-minute rest (which acted as the NIRS baseline for CBF calculations) was taken at each testing session and data were acquired continuously throughout a cognitive task battery. Output was time-stamped at each task segment and averaged over the task period. Baseline adjusted data with respect to the 5 min of NIRS data collected immediately prior to completing the tasks [44; 45], was calculated offline. NIRS data are reported as changes in cerebral oxy- (HbO_2), deoxy- (hHb) and total-(tHb) haemoglobin concentrations.

Cognitive function, mood, sleep and health assessment

Participants completed the Pittsburgh Sleep Quality Inventory, (PSQI; [46]) and a short form quality-of-life survey (SF-36; [47]) to assess sleep quality and health, respectively. 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 [22; 45]. 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 [48] 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 (%

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

Digit Vigilance (DV)

The DV task is a measure of sustained attention and psychomotor speed. 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 spacebar on the keyboard as quickly as possible every time the digit in the series matched the target digit. Task outcomes included accuracy (%) and reaction time for correct responses (ms) and number of false alarms. This task has been shown to identify age-related declines in attention and the test-retest correlation coefficient for reaction time is 0.81 [49].

Rapid Visual Information Processing (RVIP)

The RVIP task is a measure of sustained attention and working memory. The 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 spacebar on the keyboard as quickly as possible. Task outcomes included number of target strings correctly detected (%) and average reaction time for correct detections (ms) and number of false alarms. The test-retest correlation for these is (>0.70) in older adults and has been reported as reliable in the detection and monitoring of cognitive deficits [50].

N-Back

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

Metabolomics protocol

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

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

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

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

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

Statistical analysis

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

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

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

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

Results

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

The effect of MC on cerebral blood flow

After 3-month supplementation there was no treatment or treatment × epoch interaction effects for HbO_2, hHb or tHb concentrations assessed by NIRS during any of the tasks (Supplemental Figure 2).

The effect of MC on sleep and health

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

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

The effect of MC on cognitive performance and mood

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

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

The effect of MC on plasma metabolome

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

Discussion

The main finding of this study was that tart cherries have a positive impact on cognitive performance and perceptions of fatigue and alertness and upregulate plasma amino acids, with no influence on CBF, sleep or health. In the current study MC improved sustained attention measured by DV. Both sweet [29] and tart [31] cherries have been shown to improve aspects of cognitive function following 12-week supplementation in older adults, including sustained attention, however it is currently unknown whether this is a result of improved CBF or due to the potential neuroprotective properties of tart cherry anthocyanins [17]. Therefore, we measured blood flow with NIRS placed over the prefrontal cortex, but no changes in cognitive function or CBF in response to MC intake were observed. There is no directly comparable study, and hence this represents the first study to determine cognitive performance and NIRS in response to chronic supplementation of MC. Although our research group has shown that an acute bolus of MC can influence CBF [22], we did not observe any influence following chronic supplementation in the current study. This is likely to be attributable to the vasomodulatory properties of the cherries coincide with peak plasma concentrations of anthocyanin metabolites, which are rapidly metabolised and/or excreted [21]. It is therefore conceivable that changes in vascular function are relatively transient with the bioavailability of the phytonutrients and hence any possible changes from the previous day had passed. This is consistent with our finding that 3 month supplementation had no influence on vascular function variables after an overnight fast [34]. Moreover, the data it the present study are in line with previous studies that reported that both resveratrol [45] and *Sideritis scardica* [54] supplementation induced acute, but not chronic changes in CBF parameters measured by NIRS. Furthermore, in a recent 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 [55]. For instance, Bowtell
395 *et al.* [56] 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 [57; 58], 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 [59]. 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 [22], 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 [60; 61], 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

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

There were no differences found in sleep measures between groups after the intervention, as assessed by the PSQI. This contradicts previous research which showed that tart cherries, due to their melatonin content, improved sleep quality [27; 43; 70]. However, this is likely because of the use of a questionnaire in this study rather than any objective measures of sleep quality. For example, previous research reported improved sleep efficiency and total sleep time measured by actigraphy, following 7-day consumption of MC, but the same measures collected by questionnaires did not differ [27]. Importantly there were no substantial changes in the sleep patterns of 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 [34], the sugar content of
445 MC and potential variability between batches need to be carefully considered in future
446 research designs [71]. 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 [30; 72;
449 73]. 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

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

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Table 1. Nutritional composition of treatments per 60 ml 684

	MC concentrate	**Placebo**
Energy (kcal)	204	204
Carbohydrate (g)	50	51
Protein (g)	2.2	0.0
Fat (g)	0.0	0.0
TACN (mg) mean ± SD[a]	22.2 ± 6.7	-
TPC (mg) mean ± SD[b]	195.5 ± 13.1	21.5 ± 2.3

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

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

[b]Analysed using a modified Folin-Ciocalteu colorimetric method.

Table 2. Baseline characteristics of participants

Variable	**Cherry** (n = 25)	**Placebo** (n = 25)	P-Value
Age (y)	49 ± 6	47 ± 6	0.465
Sex (m/f)	8/17	8/17	
Stature (cm)	173.8 ± 9.2	173.2 ± 8.9	0.884
Body Mass (kg)	82.9 ± 13.9	82.9 ± 12.5	0.968
BMI (kg/m^2)	27.3 ± 3.8	27.5 ± 3.8	0.570
Anthocyanins (mg/day)	9.7 ± 17.2	17.8 ± 40.5	0.958
Total polyphenols (mg/day)	571.8 ± 244.9	327.9 ± 195.8	0.010
***Education* (*n*; %)**			
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)	
Left-handed (n; %)	3 (12)	1 (4)	
Regular use of medication* (n; %)	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)	-	

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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
Sleep Latency (mins)	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
Sleep Duration (mins)*	50				
Baseline		434 ± 63	398 ± 28	T	**0.015**
3 months		421 ± 54	409 ± 38	T*V	0.197
Habitual Sleep Efficiency (%)	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
Global PSQI score	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
General health (%)	49				
Baseline		67 ± 19	71 ± 18	T	0.462
3 months		68 ± 13	69 ± 15	T*V	0.569
Health change (%)	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 ± SD)

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

			Baseline		3 months		Mixed Model	
Measure	**Treatment**	n	**Rep 1**	**Rep 2**	**Rep 1**	**Rep 2**	**Effect**	**P-Value**
Alert (%)*	Cherry	48	71.8 ± 9.5	52.6 ± 14.8	69.3 ± 11.3	59.7 ± 18.5	T	**0.013**
	Placebo		71.8 ± 11.1	47.8 ± 14.2	65.6 ± 15.3	43.2 ± 14.8	T*R	0.093
Content (%)	Cherry	48	77.5 ± 9.2	68.1 ± 12.6	81.0± 7.9	74.4 ± 12.2	T	0.166
	Placebo		76.8 ± 10.9	60.5 ± 18.9	75.8 ± 13.8	66.1 ± 17.9	T*R	0.783
Calm (%)	Cherry	49	74.2 ± 9.6	57.3 ± 13.5	76.0 ± 13.1	64.7 ± 15.3	T	0.699
	Placebo		65.1 ± 18.0	54.4 ± 13.1	74.8 ± 13.7	61.8 ± 18.4	T*R	0.657
Mental fatigue (%)*	Cherry	46	53.5 ± 18.1	72.3 ± 10.5	48.8 ± 21.9	56.0 ± 22.9	T	**0.009**
	Placebo		59.0 ± 15.1	72.4 ± 21.7	56.6 ± 13.3	64.3 ± 24.6	T*R	0.257

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.