Concord Grape Juice, cognitive function and driving performance: a 12 week, placebo controlled, randomised, crossover trial in mothers of pre-teen children

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Abbreviations: CGJ Concord grape juice; MCI mild cognitive impairment; CREB cAMP-response element-binding protein; BDNF brain derived neurotrophic factor; VVLT Visual Verbal Learning Test; VSLT Visual Spatial Learning Test; RVIP Rapid Visual Information Processing; TOH Tower of Hanoi; SDLP standard deviation of lane position; TtLC time to line crossing; STREV steering reversal rate; HFS high frequency component of steering

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Abstract

Background: Daily consumption of Concord grape juice (CGJ) over three to four months has been shown to improve memory function in adults with mild cognitive impairment, and reduce blood pressure in hypertensive adults. These benefits are likely due to the high concentration of polyphenols in CGJ. Increased stress can impair cognitive function and elevate blood pressure. Thus we examined the potential beneficial effect of CGJ in individuals experiencing somewhat stressful demanding lifestyles.

Objective: To examine the effects of twelve weeks' daily consumption of CGJ on cognitive function, driving performance, and blood pressure in healthy, middle-aged working mothers.

Design: Twenty five healthy mothers of pre-teen children, aged 40-50 years, who were employed for $\geq$ 30 hours/week consumed 12oz (355ml) CGJ (containing 777mg total polyphenols) or an energy, taste and appearance matched placebo daily for twelve weeks according to a randomised, crossover design with a four week washout. Verbal and spatial memory, executive function, attention, blood pressure and mood were assessed at baseline, six weeks and twelve weeks. Immediately following the cognitive battery, a subsample of seventeen females completed a driving performance assessment in the University of Leeds Driving Simulator. The twenty five minute driving task required participants to match the speed and direction of a lead vehicle.

Results: Significant improvements in immediate spatial memory and driving performance were observed following CGJ relative to placebo. There was evidence of an enduring effect of CGJ such that participants who received CGJ in arm 1 maintained better performance in the placebo arm.

Conclusions: Cognitive benefits associated with chronic consumption of flavonoid-rich grape juice are not exclusive to adults with mild cognitive impairment. Moreover, these cognitive benefits are apparent in complex everyday tasks such as driving. Effects may persist beyond cessation of flavonoid consumption and future studies should carefully consider the length of washout within crossover designs.

Introduction

Recent reviews have suggested consumption of polyphenol-rich foods and drinks such as grape juice, blueberries and cocoa are associated with cognitive benefits in humans [1]. Concord grape juice (CGJ) is a rich source of polyphenols, particularly flavonoids such as proanthocyanidins and anthocyanins, which have been postulated to positively affect cognitive function by a variety of mechanisms including inhibition of neuroinflammation [2] and inducing neurogenesis and synaptic plasticity [3]. Improved verbal learning and reduced semantic interference on memory tasks in older adults with mild cognitive impairment (MCI) were reported following daily consumption of CGJ for twelve weeks [4] and sixteen weeks [5]. Evidence for underlying mechanisms comes from blueberry supplementation studies in aged rats, where increased activation of cAMP-response element-binding protein (CREB), increased hippocampal levels of brain derived neurotrophic factor (BDNF) and an accompanying benefit to spatial working memory were observed following three to twelve weeks of supplementation [6]. Enhanced vascular physiology in the brain such as improved cerebral blood flow has been observed in humans following consumption of cocoa flavanols [7,8]. It is proposed that such effects are mediated by enhanced endothelial function and increased bioavailability of nitric oxide [9,10]. Increased activation in the right middle prefrontal and right superior parietal cortical regions using fMRI (functional magnetic resonance imaging) was observed in adults with MCI following CGJ consumption enhanced cerebral blood flow. Endothelial and nitric oxide pathways are implicated in the beneficial effects on cardiovascular outcomes induced by CGJ and other polyphenol rich foods and drinks [11,12].

The allostatic load model describes how chronic stress, CVD and associated risk factors can lead to a cascade of physiological and psychological consequences, such as cognitive impairment [13-15]. Individuals experiencing elevated levels of daily stress may, therefore,

be particularly responsive to the benefits of consuming polyphenol-rich foods and drinks such as CGJ, as supported by animal models [16]. We recruited a healthy cohort who reported experiencing a psychologically demanding and potentially stressful lifestyle, and thus might benefit from chronic polyphenol consumption; working mothers with pre-teen children. Whilst it is important to consider effects on specific cognitive domains reflecting particular neurological processes, the ecological validity; i.e. extent to which performance on a battery of cognitive tests is related to everyday cognitive tasks is also important [17]. Driving is a highly ecological behaviour requiring a multitude of complex processes encompassing all cognitive domains. Psychologically stressed individuals are more vulnerable to driving errors due to an overload of attentional resources [18]. It is, therefore, of interest to examine whether dietary polyphenol based interventions can affect performance on everyday tasks such as driving, which can be assessed in a controlled laboratory environment. This study examined performance on several cognitive domains shown to be sensitive to berry polyphenol consumption [1], in addition to longitudinal and lateral tracking tasks within an immersive driving environment using a state of the art driving simulator.

Subjects and Methods

Design

Concord grape juice (CGJ) or a placebo were consumed daily for twelve weeks according to a double-blind randomised crossover design. Previous data indicate that twelve weeks daily consumption of CGJ is a sufficient duration to detect cognitive benefits [4]. Participants were assigned to one of two orders (CGJ then placebo, or placebo then CGJ) according to a counterbalanced randomisation schedule prepared by an independent statistician. Outcome variables were assessed at three time points per arm: baseline (prior to drink consumption),

six weeks (post daily consumption) and twelve weeks (post daily consumption). Each arm was separated by a four week washout.

Treatment Drinks

The 12oz (355ml) daily servings of CGJ and placebo were matched for energy (233kcal), appearance, taste, volume (355mls), and carbohydrate content (59.5g) and all sugars (54g). The CGJ contained 777mg total polyphenolics as gallic acid equivalent per 355ml daily serving (167mg anthocyanins as malvidin equivalent and 334mg proanthocyanidins as catechin equivalent). No vitamin C was present in either CGJ or placebo. Total polyphenol concentration was determined by the Folin-Ciocalteu procedure [19]. Anthocyanins were determined by a spectrophotometric procedure [20]. Proanthocyanidins were determined by normal-phase, high-performance liquid chromatography after solid-phase extraction of the juice with a Sephadex LH-20 [21,22]. The daily dosage was selected upon evaluation of previous research showing that a 12oz (355ml) daily dose of CGJ containing 740mg total polyphenolics over twelve weeks is sufficient to detect cognitive benefits [4]. Both drinks were analysed and prepared by Welch Foods Inc, USA and refrigerated at between 1-5^{o}C until distributed to participants who were instructed to keep the juice refrigerated. To ensure double-blinding the CGJ and placebo were labelled with a three letter code by Welch Foods Inc. The code was revealed to the experimenters on completion of the analysis. Both drinks underwent standard microbiological and safety testing by Welch Foods Inc. To examine equivalence of taste participants responded to the following question "How pleasant did you find the drink" on a 10cm Likert scale after consuming the drink at each visit.

Participants

Twenty five working female mothers were recruited from Leeds (UK) and the surrounding area. The inclusion criteria were ; 40-50 years of age, at least one child younger than 13 years

of age, BMI 18-29kg/m^2, employment $\geq$ 30 hours per week, adequate understanding of verbal and written English, possession of a full driving licence for >5 years, having driven >5000 miles in the past year. Exclusion criteria were smoking, self-reported menopausal symptoms, working night shifts, pregnant or planning pregnancy in the next six months, consuming > three portions of fruit/vegetables per day, vegetarian, current or history of eating disorder (>20 EAT-26) [23], and any current illness or disease. The following additional exclusion criteria were required for the driving performance subsample: epilepsy, claustrophobia, fear of heights and severe motion sickness. All eligible participants completed a screening test drive in the driving simulator and were familiarised with the cognitive test battery and other measures before randomisation. This required completion of a full version of the cognitive battery. Twenty five participants started arm one. Two participants withdrew after completing the first arm (n=2, both completed the placebo arm) neither of whom gave a reason for withdrawal. A further four withdrew during the second arm (n=3 CGJ and n=1 placebo). Of these four withdrawals, one reported depression (CGJ), and three reported they no longer wanted to drink the juice. Therefore, twenty five participants completed the first arm and nineteen participants completed both arms. Of the twenty five initial recruits, seventeen met the driving simulator inclusion/exclusion criteria. Cognitive performance was the primary outcome measure, and therefore, participants who failed the driving simulator screening were retained and data were collected for all other outcome measures. Of the seventeen participants who passed the driving simulator screening, one participant was excluded for non-compliance with the driving task. Of the remaining sixteen participants, driving performance data for both arms were available for eleven participants (since, as described above, five participants withdrew during arm two). Participant characteristics at screening are show in **Table 1**.

[Table 1 here]

Cognitive Function

The 45 minute cognitive test battery comprised of seven tests administered in the following order; Visual Verbal Learning Test (VVLT) immediate recall (verbal memory), Visual Spatial Learning Test (VSLT) immediate recall (non-verbal, spatial memory), Rapid Visual Information Processing (RVIP) (executive function), Grooved Peg Board (psychomotor skill), Tower of Hanoi (TOH) (executive function), VVLT delayed recall and VSLT delayed recall. Equivalent versions of each test were incorporated in a counterbalanced order across the six cognitive test sessions, whilst the order in which the tests occurred within the cognitive test battery remained constant across all administrations.

The Visual Verbal Learning Test (VVLT) is a visual analogue of the Rey Auditory-Verbal Learning Test [24]. Three trials of sixteen words (List A) were presented in a random sequence on a computer screen at the rate of one word every two seconds. At the end of each trial, participants were instructed to verbally recall as many of the words as possible in a free recall task over one minute (trials A1, A2 & A3). Trial A3 was followed by presentation of a sixteen word interference list (List B) and a subsequent free recall of these words over one minute (trial B1). A one minute free recall of List A without presentation immediately succeeded this (trial A4). Outcome variables were the mean number of words recalled over trials A1-A3, retroactive interference (A3-A4) and proactive interference (A1-B1). Delayed memory was assessed thirty minutes after the initial presentation of the test.

The Visual Spatial Learning Test (VSLT) [25] is a test of visuospatial memory and learning. The original version of the test was designed as an assessment of dementia and involves five trials. However, to avoid ceiling effects in the present sample without dementia only three trials were administered. At each identical trial participants were given ten seconds to observe the image and location of seven abstract patterns placed on 5x4 grid. The task was to

correctly choose the seven target patterns from a choice of fifteen and place them on the correct locations on the grid. The outcome variable was the number of correct targets placed in the correct location per trial (maximum 7). Delayed VSLT performance was assessed thirty minutes after initial presentation of the test.

The RVIP involved presentation of a series of single digits at a rate of 600 milliseconds with a 600 millisecond inter-stimulus interval. The task lasted for six minutes with 100 stimuli per one minute block and five odd and five even targets within each block. Target sequences were three consecutively presented odd or even numbers which required participants to press the space bar as quickly as possible. Outcome variables were the number of correctly identified sequences, the number of false positive responses and reaction time for correct responses.

The Grooved Peg Board [26] assesses manual dexterity and is a test of psychomotor skill. The apparatus consisted of a board with 25 holes and 35 identical pegs. The pegs had a groove down one side. The holes were to be filled with pegs in a specified order as quickly as possible. Participants completed the task with their dominant hand followed by their non-dominant hand. The outcome variable was completion time to fill the board with pegs (averaged across both hands).

The Tower of Hanoi [27] is a test of planning ability which is considered an exemplar measure of executive function. A computerised version was administered. This consisted of a visual representation of three rods upon which four discs of different size and colour were placed. At the top of the screen was the target formation of discs on the rods and at the bottom of the screen was the starting formation. The aim of the task was to rearrange the disks on the starting formation rods to match the target formation in the fewest possible moves. There was only one correct sequence of moves for each trial. This was the fewest

number of moves required to match the target formation. If the participant deviated from the correct sequence the screen refreshed to the original starting formation. There was one rule: a disk could not be placed on a disk which was larger than itself, such moves were not recorded as errors as the computer would not allow it. At the outset, the screen informed the participant of the number of moves required to complete a trial. There were ten trials per test administration, consisting of two trials for each of the five levels of 4,5,6,7 and 8 moves. Level 4 trials consisted of a sequence of four blocks, level 5 consisted of a sequence of five blocks, and so on. There were two outcome variables: (i) number of errors made, and (ii) completion time.

Blood pressure and subjective mood

Resting systolic and diastolic blood pressure were measured with an ambulatory blood pressure monitor (Omron M7). Measurements were taken following 15 minutes rest on the left upper arm, and the average of three consecutive measurements was calculated. Subjective mood was assessed using pen and paper 100mm Visual Analogue Scales (VAS) with questions relating to hunger, fullness, contentedness, irritability, sleepiness, mental alertness, ability to concentrate, and energy with anchors for each adjective; “not at all” and “very”. Subjective stress was assessed with the Perceived Stress Scale (PSS) and state anxiety was assessed using the short 6-item form (MSTAIY6) [28] of the State Trait Anxiety Inventory [29].

Driving Performance

A twenty five minute virtual driving scenario of car following and lane keeping was choreographed within the University of Leeds Advanced Driving Simulator. The high fidelity simulator consists of a Jaguar S-type vehicle, a 4m diameter spherical projection dome which houses an eleven channel display system covering a forward field of view of 270° and a large

amplitude, eight degree-of-freedom electrical motion system with five meters of effective travel in surge and sway. Driving performance was assessed by two tasks; (i) a longitudinal task (car following) and (ii) a lateral task (lane keeping). The longitudinal and lateral tasks were performed concurrently.

The longitudinal task required participants to "follow a lead vehicle at a safe and constant distance gap". The lead vehicle adjusted its speed in a smooth fashion matching a 60 second period sine wave with a maximum speed of 60mph and a minimum speed of 40mph until five cycles of the sine wave were completed (a driving distance of approximately seven km). The drive consisted of sections of straight and curved road. The curved sections were alternate bends to the left and right with a radius of 750 meters and a length of 252 meters. There were three independent variables for the longitudinal task; coherence, phase shift and modulus [30]. Coherence, a measure of correlation of the two speed cycles, indicates the accuracy of the participant's speed adjustments in order to meet the main longitudinal tracking task requirement to maintain a consistent following distance. Coherence can range from zero (no coherence between the two cycles) to one (perfect coherence). Phase shift measures the delay between the change in the lead vehicle's speed and the subsequent response (akin to a delay in response). Modulus is an amplification factor between the two signals, expressed as the amplitude gain; if the participant overreacts to the lead vehicle's speed changes, the modulus will be larger than one. Data was analysed for the final four of the five, five minute sine wave cycle; the first cycle allowed the participant time to settle into the task.

The lateral task required participants to maintain a constant position in the driving lane. There were four outcome measures for the lateral task; standard deviation of lane position (SDLP), time to line crossing (TtLC), steering reversal rate (STREV) and high frequency component of steering (HFS). The SDLP measured the variation in lane position. TtLC was defined as the time to cross either lane boundary with any of the wheels of the vehicle. Therefore, as the

vehicle approached the edge of the road, TtLC decreased. A TtLC <2 seconds was the threshold for close proximity to exiting the lane boundary [31]. A TtLC < 2 seconds is termed TETtLC2. The total time exposure during which TETtLC2 occurred was measured. Therefore, TETtLC2 defined the duration for which the participant was driving in close proximity to the lane boundary. STREV was the number of changes in steering wheel direction per minute (an angle of 1^{o} is required to qualify as a reversal). A higher STREV indicated higher difficulty in achieving accurate tracking. HFS reflected the number of steering corrections. Increased corrections were indicative of erratic, reactive driving as opposed to having a strong awareness of the road ahead which is associated with predictive steering and fewer corrections [32].

Procedure

Inclusion and exclusion criteria were checked at a screening session which took place at the University of Leeds, Human Appetite Research Unit (as did all test days and procedures notwithstanding the driving simulator procedures which took place at the University of Leeds Advanced Driving Simulator). Screening included a recruitment information questionnaire (assessing general health), a measure of eating behaviour (EAT-26) the National Adult Reading Test (NART) [33], baseline PSS, a measure of hostility (Cook-Medley Hostility Scale) [34], and a measure of baseline trait anxiety (Spielberger State-Trait Anxiety Inventory, STAIY2 [29]. Height, weight and blood pressure were measured and familiarisation versions of the cognitive battery and driving performance task were completed. Participants were informed that consumption of the following polyphenol-rich drinks were not permitted for the duration of the study, including the four week washout; red wine, grape juice (notwithstanding the treatment drink), or any dark fruit juices. A minimum of one week separated the screening visit and test day 1. The day before each test day, participants refrained from exercise and alcohol consumption after 17:00 hours, and were told

to consume an evening meal of their choice before 21:00 hours. The meal was standardised across all test days (within participants) to control for second meal cognitive effects [35]. All test days commenced between 07:30 and 10:00 hours with participants fasted (except water) from 21:00 hours. Data were collected in the following order; weight, mood #1 (VAS), stress (PSS), anxiety (MSTAIY6) and blood pressure. The treatment drink was then consumed within fifteen minutes followed by mood #2 and commencement of the cognitive battery. Mood#3 was assessed upon completion of the cognitive battery and participants were escorted to the Institute for Transport Studies to commence the driving performance task. Immediately prior to and after the driving task mood #4 and #5 were assessed. The total time for the test day was approximately two hours. To monitor compliance, participants were required to return bottle tops from the consumed drinks to the Human Appetite Research Unit on a weekly basis and to complete a daily drink diary detailing time of consumption. Compliance was high; 22 participants reported 100% compliance, and all participants except one exceeded 90% compliance (at least 75 days recorded). The lowest compliance was 76% (64/84 days recorded). Participants received a £160 honorarium upon completion of the study, or pro rata for withdrawals. The School of Psychology Research Ethics Committee reviewed the procedures and awarded a favourable opinion for conduct. Recruitment commenced in January 2011 with the last participant completing in June 2012.

Statistical Analysis

Analysis was performed by an independent statistician. An intention to treat (ITT) analysis was performed which included data for all participants who completed the first arm (n=25 for cognitive performance, and n=16 for driving performance). Within-subjects ANCOVAs were performed to examine the effects of Condition (CGJ/placebo) and Visit (day 1 (baseline), 6 weeks, 12 weeks) nested within Study Phase (arm 1/arm 2) on all outcome variables. Covariates were age, IQ (NART), subjective stress (PSS), psychological hostility (Cook-

Medley Hostility Scale), state anxiety (STAIY2) and eating behaviour (EAT-26), all of which were assessed at screening (see procedure).

All main effects and their interactions were requested in the first model and all covariates were included. Non-significant interactions with covariates were removed first and the analysis was re-run. Next, non-significant covariates were removed, then higher order interactions of fixed factors. The resulting model was compared to the previous model using the McQuarrie Tsai AICc criterion [36]. The AICc criterion gives an indication of the amount of remaining unexplained variance after the model has been fitted, where a smaller AICc value indicates a better model. If an improvement in model fit was found, other non-significant effects were removed and again the AICc criterion used to evaluate the model fit. Models were chosen on the basis of 'best fit', and interaction terms that improved the fit were retained. The reported ANCOVAs are the 'best fit' (i.e. lowest AICc) models adjusted for significant covariates. Tukey-Kramer post hoc tests were employed to follow-up significant main effects or interactions and the p-values reported are adjusted for multiple comparisons. Analyses were performed with SAS® v9.3 and all residuals were screened and outliers were removed.

Results

Drink Characteristics

The ANCOVA for "pleasantness" data showed no significant main effects or interactions, indicating equivalence of taste. Upon completion of both arms 12/19 (63%) participants identified CGJ as the active drink, which was not significantly different to chance according to a one sample t-test ($t=1.16$, $df=18$, $p=0.26$).

Cognitive Performance

316 Verbal recall

317 VVLT immediate recall averaged over the first three trials (A1 to A3) showed a significant
318 Condition*Study Phase interaction ($F[1,20]=4.61$, $p<0.05$). As shown in **Figure 1**, post hoc
319 tests revealed that this interaction was specific to the placebo condition whereby recall was
320 better when the placebo was consumed in arm 2 relative to arm 1 ($p<0.05$). This indicates an
321 initial benefit of the CGJ in arm 1 which endured into arm 2 when the placebo was
322 consumed. No significant effects were observed for retroactive, proactive interference, or
323 delayed VVLT recall (see **Table 2**).

324 Spatial Recall

325 VSLT immediate recall showed a significant main effect of Condition ($F[1,22]=5.58$, $p<0.05$)
326 such that recall (total over 3 trials) was higher following CGJ (mean 12.72, se 0.39 relative to
327 placebo (mean 12.57, se 0.36). Immediate and delayed VSLT recall were significantly higher
328 in arm 2 relative to arm 1 as indicated by a main effect of Study Phase (immediate
329 $F[1,21]=12.8$, $p<0.01$; delayed $F[1,17]=5.24$, $p<0.05$).

330 Psychomotor skill

331 Completion time for the Pegboard showed a significant Condition*Study Phase interaction
332 ($F[1,21]=9.61$, $p<0.01$). Post hoc tests revealed that in arm 1, completion time was
333 significantly faster for the placebo (mean 60.4 secs se 1.9) relative to CGJ (mean 63.2 se 2.3)
334 ($p<0.05$) whereas no difference was observed between the two drink conditions in arm 2.

335 Executive function

336 ToH completion time showed a significant Condition*Study Phase interaction
337 ($F[1,21]=14.12$, $p<0.01$). As shown in **Figure 2**, post hoc tests revealed that completion time
338 was significantly faster for the CGJ relative to the placebo in arm 1 ($p<0.01$) whereas this

difference was not significant in arm 2. A main effect of Study Phase was observed for the number of errors ($F[1,21]=5.27$, $p<0.05$) such that fewer errors were made in arm 2 (mean 3.6 se 0.6) relative to arm 1 (mean 6 se 1.1). No significant effects were observed for reaction time, correctly identified targets or false positives on the RVIP (see Table 2).

[Figures 1 & 2 here]

Driving Performance

Longitudinal tracking task (car following)

Analysis of coherence revealed that the main effect of Condition was significant ($F[1,11]=4.64$, $p=0.05$) such that car following was more accurate during CGJ (mean 0.97 se 0.01) relative to placebo (mean 0.96 se 0.01). Similarly, the main effect of Condition approached significance for phase shift analysis ($F[1,11]=4.26$, $p=0.06$) such that CGJ was associated with a quicker driver response to changes in the lead vehicle speed (CGJ mean 3.54 se 0.54; placebo mean 4.13 se 0.64). Finally, analysis of modulus revealed a main effect of Study Phase ($F[1,10]=6.67$, $p<0.05$) such that performance was better (less overshoot) in arm 1 (mean=1.1 se 0.03) relative to arm 2 (mean 1.14 se 0.03).

Lateral tracking

A significant Condition*Study Phase interaction was observed for steering reversals ($F[1,10]=16.73$, $p<0.01$) such that during arm 1 steering control was more stable (fewer steering reversals) for the CGJ condition, whereas at arm 2 steering control was more stable for the placebo condition (although post hoc tests did not reach significance; see **Figure 3**). No significant effects were observed for time to line crossing (TETtLC2), lane position (SDLP) or for high frequency component of steering (HFS).

[Figure 3 here]

Subjective Outcomes & Blood Pressure

Contentedness was significantly higher in arm 1 (mean 64 se 2.6) relative to arm 2 (mean 60 se 2.5) as indicated by a main effect of Study Phase ($F[1,21]=5.35$ $p<0.05$). Alertness and concentration were significantly higher for participants who consumed CGJ in arm 1; mean 58 and 60 respectively (se 3.6 and 3.7), relative to participants who consumed CGJ in arm 2; mean 53 and 56 respectively (se 3.4 and 3.3) (both $p<0.05$) as indicated by significant Condition*Study Phase interactions ($F[1,21]=19.45$, $p<0.001$ & $F[1,21]=24.98$, $p<0.0001$ respectively). A significant Condition*Study Phase interaction was also observed for stress ratings ($F[1,21]=4.86$, $p<0.05$), however, post hoc tests failed to reach significance. There were no other significant effects of interest for the subjective mood outcomes. There were no significant effects for either diastolic or systolic blood pressure (see Table 2).

[Table 2 here]

Discussion

CGJ was associated with better immediate spatial memory and two aspects of driving performance in this sample of healthy working mothers of pre-teen children aged 40-50 years, relative to a placebo. Previous interventions demonstrating benefits of CGJ have been performed exclusively in older adults with mild cognitive impairment [4,5], thus this is the first study to demonstrate cognitive benefits following CGJ in healthy middle-aged adults. These results in combination with another recent study showing executive function and memory improvements following consumption of flavanone-rich orange juice [37] indicate cognitive benefits achieved from regular daily flavonoid consumption are not exclusive to adults exhibiting cognitive decline or neurodegenerative disease. Moreover, long term-consumption of CGJ was associated with reduced phase shift in car following, comparable to quicker reaction time to unfolding traffic events. This was evidenced by increased steering

accuracy in combination with a faster response time to changes in lead vehicle behaviour during car following. The observed effects would account for a reduction in stopping distance of approximately eleven meters at the speeds driven (40-60mph), which is a significant safety benefit. Thus, the cognitive effects of chronic CGJ consumption translate into meaningful outcomes on everyday tasks.

The observed subtle benefit for spatial memory is consistent with rodent literature [6,38] and several other polyphenol interventions in humans. It has been proposed that memory function is particularly susceptible to potential mechanisms which underlie the association between flavonoids and cognitive benefits [3], possibly because the hippocampus is a region where flavonoids and their metabolites seem able to exert their actions [6,38]. In support, improved performance of a spatial recognition task and increased activation using fMRI was seen in the dentate gyrus (a sub-region of the hippocampus) in healthy older adults following 3 months consumption of flavanol-rich cocoa [7].

The consistent interaction between drink condition and study phase in this study over a number of cognitive outcomes, suggests enduring effects of CGJ following cessation of consumption. Specifically, benefits for verbal recall, executive function and lateral tracking associated with CGJ consumption in arm one persisted into the second arm when the placebo was consumed. Similar enduring effects were recently reported following eight week consumption of flavanone-rich orange juice after a four week washout on tests of executive function and memory [37]. These enduring effects may signify that polyphenols cause relatively stable physiological effects which do not dissipate rapidly after withdrawal from the diet. This has implications for determining a suitable washout period between treatments; 4 weeks may not be sufficient in flavonoid interventions. It is also noteworthy that persistent main effects of study phase revealed performance was significantly better at the second arm

regardless of the drink consumed (e.g. immediate and delayed spatial memory, executive function). This is indicative of practice effects whereby participants improved over time. Given that cognitive benefits in healthy populations are likely to be small [17], it is crucial that potential practice effects are considered by including study phase and drink order within statistical models examining cognitive effects of flavonoid interventions. This study phase effect was not exclusive to objective cognitive outcomes; higher ratings of alertness and concentration were reported during the first arm relative to the second arm (albeit only for the CGJ condition). It is perhaps not surprising that the alertness and conscientiousness of participants may decline over the course of a twenty eight week trial, and it is entirely possible that these subjective effects have an impact upon cognitive performance [39]. This further emphasises the importance of considering order effects in chronic interventions. Previous studies which reported strong effects of CGJ on immediate verbal memory [4] and spatial recognition [5] adopted a parallel groups design where order effects are avoided.

The present data show that grape flavonoids may improve performance on everyday tasks; safer driving behaviour was observed following the CGJ relative to the placebo as indicated by characteristics such as a faster response to the lead vehicle. The potential for flavonoid-rich diets to have a small but significant impact on cognitive tasks such as driving should not be overlooked, and future research should consider effects on other ecologically valid every-day tasks throughout the lifespan. Acute benefits of flavonoid consumption for the peripheral vascular system in the immediate postprandial period are well documented [10]. Several studies have shown improvements in endothelial function in patients with heart disease following grape juice supplementation [40,41] however, the present data did not show any chronic effects of CGJ on blood pressure. This could be a function of the population; the sample of healthy middle aged females did not have high blood pressure at the outset and

434 therefore the potential for a relatively short dietary intervention to have a significant impact is
435 limited.

436 It is important to point out that during this 28 week trial participants did not abstain from all
437 dietary sources of anthocyanins and other polyphenols. The specific restrictions included red
438 wine, dark fruit juice and any other grape juice. It is possible that the participants consumed
439 other sources of polyphenols which may have masked the effectiveness of the treatment
440 drink. However, high habitual polyphenol consumers were likely to have been captured by
441 the exclusion criterion of >3 portions of fruits/veg per day, and it is unlikely that the habitual
442 diets of the participants would have varied significantly between the two arms of the study. A
443 strength of the present data is its generalisability; effects were observed regardless of any
444 variability in habitual polyphenol intake, and in the context of the participants' normal diets.
445 Future studies should assess habitual intake with food diary records together with urinary
446 measures of biomarkers and metabolites which also provide evidence of compliance to
447 treatment. Finally, in light of the observed carry over effects, researchers should consider
448 incorporating a suitable washout period prior to commencement of similar polyphenol
449 interventions (and between arms), in order to reduce effects of habitual polyphenol intake,
450 thus allowing a more sensitive assessment of the intervention. Presently, habitual dietary
451 polyphenol intake prior to trial commencement and during the trial could account for some of
452 the variance indicated by the Condition*Study Phase interaction for verbal recall.

453 **5. Conclusions**

454 In summary, twelve week consumption of CGJ was associated with subtle improvements in
455 immediate spatial memory and safer driving behaviour relative to the placebo in this sample
456 of healthy working mothers of pre-teen children aged 40-50. This is the first study to
457 demonstrate benefits of CGJ in healthy adults on domain specific and everyday cognitive

tasks such as driving. However, it is important to acknowledge that there were no effects on the majority of cognitive outcomes. Importantly, there was evidence for enduring flavonoid effects such that when CGJ was consumed during the first arm some of the associated benefits persisted into the second arm when the placebo was consumed. In addition, practice effects were observed such that performance was generally better during the second arm, regardless of condition. The combination of the enduring effects and the practice effects may have masked some of the potential effects of the CGJ, particularly in this healthy middle-aged sample in whom effects of nutritional interventions are likely to be small. These findings have clear implications for the design of future crossover interventions, and indicate that the cognitive effects of CGJ are not exclusive to older adults and adults with neurodegenerative disease. Future studies should seek to explore the strength and length of cognitive effects following cessation of flavonoid supplementation.

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Conflicts of Interest

JW is an employee of Welch Foods Inc. The authors have no other conflicts of interest to declare.

References

1. Lamport DJ, Dye L, Wightman JD, Lawton, CL. The effects of flavonoid and other polyphenol consumption on cognitive performance: A systematic research review of human experimental and epidemiological studies. Nutrition and Aging 2012;1:5-25.

2. Spencer JPE, Vafeiadou K, Williams RJ, Vauzour D. Neuroinflammation: Modulation by flavonoids and mechanisms of action. Mol Aspects Med 2012;33:83-97.

3. Spencer JPE. Beyond antioxidants: the cellular and molecular interactions of flavonoids and how these underpin their actions on the brain. Proc Nutr Soc 2010;60:244-60.

4. Krikorian R, Nash TA, Shidler MD, Shukitt-Hale B, Joseph JA. Concord grape juice supplementation improves memory function in older adults with mild cognitive impairment. Br J Nutr 2010;103:730-734.

5. Krikorian R, Boespflug EL, Fleck DE, Stein AL, Wightman JD, Shidler MD, Sadat-Hossieny S. Concord grape juice supplementation and neurocognitive function in human aging. J Agric Food Chem 2012;60:5736-5742.

6. Williams CM, El Mohsen MA, Vauzour D, Butler LT, Ellis JA, Whiteman M, Spencer JPE. Blueberry-induced changes in spatial working memory correlate with changes in hippocampal CREB phosphorylation and BDNF levels. Free Radic Biol Med 2008;45:295-305.

7. Brickman AM, Khan UA, Provenzano FA, Yeung L-K, Suzuki W, Schroeter H, Wall M. Sloan RP, Small SA. Enhancing dentate gyrus function with dietary flavanols improves cognition in older adults. Nat Neurosci 2014;17:1798-1803.

499 8. Francis S, Head K, Morris PG, Macdonald IA. The effect of flavanol-rich cocoa on the
500 fMRI response to a cognitive task in healthy young people. J Cardiovasc Pharmacol
501 2006;47:S215-220.

502 9. Fisher ND, Hollenberg NK. Aging and vascular responses to flavanol-rich cocoa. J
503 Hypertens 2006;24:1575-1580.

504 10. Schroeter H, Heiss C, Balzer J, Kleinbongard P, Keen CL, Hollenberg NK, Sies H, Kwik-
505 Uribe C, Schmitz HH, Kelm M. (-)-Epicatechin mediates beneficial effects of flavanol-rich
506 cocoa on vascular function in humans. Proc Natl Acad Sci 2006;103:1024-1029.

507 11. Wightman JD, Heuberger RA. Effect of grape and other berries on cardiovascular health.
508 J Sci Food Agric 2014; doi: 10.1002/jsfa.6890.

509 12. Barona J, Blesso CN, Anderson CJ, Youngki P, Lee J, Fernandez ML. Grape
510 consumption increases anti-inflammatory markers and upregulates peripheral nitric oxide
511 synthase in the absence of dyslipidemias in men with metabolic syndrome. Nutrients
512 2012;4:1945-1957.

513 13. McEwen BS, Sapolsky RM. Stress and cognitive function. Curr Opin Neurobiol
514 1995;5:205-216.

515 14. McEwen BS. Stress and hippocampal plasticity. Ann Rev Neurosci 1999;22:105-122.

516 15. Juster RP, McEwen BS, Lupien SJ. Allostatic load biomarkers of chronic stress and
517 impact on health and cognition. Neurosci Biobehav Rev 2009;35:2-16.

518 16. Patki G, Allam FH, Atrooz F, Dao AT, Solanki N, Chugh G, Asghar M, Jafri F, Bohat R,
519 Alkadhi KA, et al. Grape powder intake prevents overiectomy-induced anxiety behaviour,

520 memory impairment and high blood pressure in female Wistar rats. PLoS One
521 2013;9:e74522.

522 17. de Jager CA, Dye L, de Bruin EA, Butler L, Fletcher J, Lamport DJ, Latulippe ME,
523 Spencer JPE, Wesnes K. Cognitive function: criteria for validation and selction of cognitive
524 tests for investigating the effects of foods and nutrients. Nutr Rev 2014;72:162-179.

525 18. Matthews G, Sparkes TJ, Bygrave HM. Stress, attentional overload and simulated driving
526 performance. Hum Perform 1996;9:77-101.

527 19. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-
528 phosphotungstic acid reagents. Am J Enol Vitic 1965;16:144-158.

529 20. Niketic-Alesksic GK, Hrazdina G. Quantitative analysis of the anthocyanin content in
530 grape juices and wines. Lebensm Wiss Technol 1972;5:163-165.

531 21. Adamson GE, Lazarus SA, Mitchell AE, Prior RL, Cao G, Jacobs PH, Kremers BG,
532 Hammerstone JF, Rucker RB, Ritter KA, et al. HPLC method for the quantification of
533 procyanidins in cocoa and chocoloate samples and correlation to total antioxidant capacity. J
534 Agric Food Chem 1999;47:4184-4118.

535 22. Gu L, Kelm M, Hammerstone JF, Beecher G, Cunningham D, Vannozzi S, Prior RL.
536 Fractionation of polymeric procyanidins from lowbush blueberry and quantification of
537 procyanidins in selected foods with an optimized normal-phase HPLC-MS fluorescent
538 detection method. J Agric Food Chem 2002;50:4852-4860.

539 23. Garner DM, Garfinkel PE. The eating attitudes test: an index of the symptoms of anorexia
540 nervosa. Psychol Med 1979;9:273-279.

541 24. Rey A, 1964. L'examen clinique en psychologie. Paris: Presses Universitaires de France

542 25. Malec JF, Ivnik RJ, Smith GE. Visual Spatial Learning Test. Psychol Assessment
543 1991;3:82-88.

544 26. Klove H. 1963. Clinical neuropsychology. In *The Medical Clinics of North America*.
545 Forster FM, Ed. Saunders: New York.

546 27. Simon HA. The functional equivalence of problem solving skills. Cognitive Psychol
547 1975;7:268-288.

548 28. Marteau TM, Bekker H. The development of a six-item short-form of the state scale of
549 the Spielberger State-Trait Anxiety Inventory (STAI). Br J Clin Psych 1992;31:301-306.

550 29. Speilberger CD. 1983. Manual for the State-Trait Anxiety Inventory. Palo Alto, CA:
551 Consulting Psychologist Press.

552 30. Brookhuis K, De Waard D, Mulder B. Measuring driving performance by car following
553 in traffic. Ergonomics 1994;37:427-434.

554 31. Godthelp H, Konings H. 1991. Levels of steering control; some notes on the time-to-line
555 crossing concept. In Proceedings of the first European Annual Conference on Human
556 Decision and Manual Control. Technical University, Delft, Netherlands.

557 32. MacDonald WA, Hoffman ER. Review of relationships between steering wheel reversal
558 rate and driving task demand. Human Factors 1980;22:733-739.

559 33. Nelson HE. 1982. National Adult Reading Test (NART): Test Manual. NFER: Nelson.

560 34. Cook WW, Medley DM. Proposed hostility and pharisaic-virtue scales for the MMPI. J
561 Appl Psychol 1954;38:414-418.

562 35. Lamport DJ, Hoyle E, Lawton CL, Mansfield MW, Dye L. Evidence for a second meal
563 cognitive effect: glycaemic responses to high and low glycaemic index meals are associated
564 with cognitive the following morning. Nutr Neurosci 2011;14: 66-72.

565 36. McQuarrie AD, Tsai CL. 1998. Regression and Time Series Model Selection. World
566 Scientific; Singapore.

567 37. Kean RJ, Lamport DJ, Dodd GF, Freeman JE, Williams CM, Ellis JA, Butler LT, Spencer
568 JPE. Chronic consumption of flavanone-rich orange juice is associated with cognitive
569 benefits: an 8-wk, randomized, double-blind, placebo-controlled trial in healthy adults. Am J
570 Clin Nutr 2015 doi: 10.3945/ajcn.114.088518

571 38. Rendeiro C, Vauzour D, Rattray M, Waffo-Teguo P, Merillon JM, Butler LT, Williams,
572 CM, Spencer JPE. Dietary levels of pure flavonoids improve spatial memory performance
573 and increase hippocampal brain derived neurotrophic factor. PLoS One 2013;28:e63535.

574 39. Hamer M, Dye L, Mitchell S, Laye S, Saunders C, Boyle N, Schuermans J, Sijben J.
575 Examining techniques for measuring the effects of nutrients on subjective mental
576 performance and mood states. Eur J Nutr, *in press* DOI: 10.1007/s00394-015-1143-3.

577 40. Stein JH, Keevil JG, Wiebe DA, Aeschlimann S, Folts JD. Purple grape juice imporves
578 endothelial function and reduces susceptibility of LDL cholesterol to oxidation in patients
579 with coronary artery disease. Circulation 1999;100:1050-1055.

580 41. Chou EJ, Keevil JG, Aeschlimann S, Wiebe DA, Folts JD, Stein JH. Effect of ingestion
581 of purple grape juice on endothelial function in patients with coronary heart disease. Am J
582 Cardiol 2001;88:553-555.

583 Table 1: Participant characteristics at baseline. Data presented are means (SE)

	All participants		**Participants undertaking the driving performance task**	
	ITT[1]	**Completed**	**ITT[1]**	**Completed**
	n=25	n=19	n=16	n=10
Age (yrs)	43.2 (.6)	42.8 (.7)	42.6 (.7)	42.5 (.9)
Weight (kg)	67.5 (1.9)	68.4 (2.3)	68.8 (2.5)	72.2 (3.8)
Height (m)	1.7 (.01)	1.7 (.02)	1.7 (.02)	1.7 (.02)
BMI (kg/m^2)	24.6 (.5)	24.4 (.5)	24.8 (.6)	25 (.7)
Systolic BP (mmHg)	127.7 (2.1)	127.9 (2.8)	129.4 (2.6)	127.7 (2.9)
Diastolic BP (mmHg)	82.6 (2.7)	82.6 (3.5)	86.2 (3.5)	82.6 (3.6)
NART[2] (max 50)	37.4 (1.6)	38.6 (1.7)	35.7 (2.2)	37.4 (3.1)
Stress (PSS[3])	14.9 (1)	15.3 (1.2)	14.1 (1.3)	15 (1.8)
Cook-Medley Hostility	14.9 (1.5)	13.8 (1.8)	15.3 (2)	16.4 (2.9)
Train Anxiety (STAI-Y2)[4]	41.8 (1.8)	41.5 (2.3)	41.6 (2.4)	42 (3.5)
Eating Behaviour (EAT-26)[5]	5.1 (1.1)	5.6 (1.4)	5.2 (1.5)	5.3 (2.3)
Pre-teen children (n)	1.6 (.2)	1.6 (.2)	1.5 (.2)	1.5 (.2)

584 1Intention to treat: This includes participants who withdrew during arm two

585 2National Adult Reading Test

586 3Perceived Stress Scale

587 4Spielberger State-Trait Anxiety Inventory

588 5Eating Attitudes Test

589

Table 2: Scores for each cognitive test outcome, driving performance and blood pressure at each test visit by condition. Data presented are means (SE)

Outcome	Placebo baseline (n=25)	Placebo six wks (n=24)	Placebo twelve wks (n=24)	CGJ baseline (n=23)	CGJ six wks (n=23)	CGJ twelve wks (n=20)	Significance and effect observed (all p<0.05)
VVLT[1] immediate recall (sum A1-A3)	33.8 (1.1)	34.8 (1.3)	35.4 (1.3)	34 (1.3)	36.2 (1.2)	36.2 (1.6)	Condition*Study Phase
VVLT retroactive interference (%)[2]	19.9 (3.1)	13.6 (2.5)	15.9 (4.7)	12.6 (2.9)	11.6 (2.3)	15.8 (6)	-
VVLT proactive interference (%)[3]	8.7 (7.6)	2.6 (5.4)	13.3 (5)	3.9 (6.9)	16.6 (5.9)	18.2 (5.8)	-
VVLT delayed recall (max 16)	10.6 (0.5)	11.6 (0.5)	11.6 (0.6)	11.3 (0.6)	11.9 (0.5)	11.8 (0.7)	-
VSLT[4] immediate recall (sum 3 trials)	12.4 (1.2)	12.4 (1)	13 (1.1)	11.3 (1.1)	12.2 (1.3)	14.1 (1.2)	Condition
VSLT delayed recall (max 7)	4.6 (0.4)	5.1 (0.4)	4.7 (0.5)	4.7 (0.4)	5 (0.5)	5.5 (0.4)	Study Phase
RVIP[5] correct (max 60)	27.5 (2.2)	29.4 (2.7)	29 (2.4)	27.6 (2.6)	29.3 (2.4)	29.9 (2.8)	-
RVIP false positives	7.3 (1.2)	6.3 (1.6)	7.3 (1.4)	8.5 (1.6)	7.5 (1.2)	6.2 (1)	-
RVIP reaction time (ms)	458 (12)	457 (18)	452 (14)	454 (16)	459 (13)	461 (16)	-
Grooved peg board completion time (sec)	62.6 (2.3)	60 (2.1)	60.6 (2)	61.2 (2.5)	61 (2.1)	59.3 (2.1)	Condition*Study Phase

Tower of Hanoi errors	5.6 (1.4)	5.1 (1.1)	4.5 (1)	4.4 (0.7)	5.5 (1)	4.1 (0.6)	Study Phase
Tower of Hanoi completion time (secs)	246 (22)	240 (17)	213 (11)	224 (11)	240 (25)	210 (11)	Condition*Study Phase
Driving coherence[6]	0.97 (0.009)	0.95 (0.011)	0.95 (0.008)	0.97 (0.009)	0.97 (0.016)	0.97 (0.013)	Condition
Driving phase shift (secs)[7]	4 (0.5)	4.5 (0.8)	3.9 (0.6)	3.7 (0.7)	3.6 (0.4)	3.3 (0.5)	Condition (p=0.06)
Driving modulus[8]	1.13 (0.04)	1.09 (0.05)	1.09 (0.04)	1.1 (0.03)	1.13 (0.02)	1.14 (0.02)	Study Phase
Driving lane position deviation[9]	0.19 (0.01)	0.18 (0.009)	0.18 (0.01)	0.18 (0.011)	0.19 (0.014)	0.18 (0.012)	-
Driving exposure to lane departure[10] (%)	30.5 (1.1)	32.6 (1.7)	31.4 (1.1)	30.7 (1)	31.3 (1.3)	31.1 (1.5)	-
Driving steering reversals (per minute)[11]	26.5 (2.8)	24.9 (1.9)	25.4 (2.4)	26 (2.2)	23.8 (1.8)	26.4 (2.5)	Condition*Study Phase
Driving high frequency steering[12]	0.25 (0.01)	0.25 (0.01)	0.25 (0.012)	0.25 (0.012)	0.25 (0.009)	0.24 (0.008)	-
Systolic blood pressure (mmHg)	122 (2.2)	117 (3.3)	120 (3.2)	120 (2.2)	124 (3)	119 (3.2)	-
Diastolic blood pressure (mmHg)	77 (1.7)	78 (2.8)	79 (3)	81 (1.9)	82 (2.9)	76 (2.7)	-

1 Visual Verbal Learning Test; 2 (number of words recalled at trial A3 – words recalled at A4) as a percentage of the number of words recalled at A3; 3 (number of words recalled at trial A1 – words recalled at B1) as a percentage of the number of words recalled at A1; 4 Visual Spatial Learning Test, 5 Rapid Visual Information Processing; 6 Coherence is a correlation between the speed cycle of the participant and the lead vehicle ranging from zero (no coherence between the two cycles) to one (perfect coherence); 7 Phase shift measures the delay between the change in the lead vehicle's speed and the subsequent response by the participant. A lower score indicates better performance; 8 If the participant overreacts to the lead vehicle's speed changes, the modulus will be larger than one; 9 Standard deviation of the lane position; 10 Exposure to imminent lane departure also known as time to line cross (TETtLC2); 11 Driving steering reversals (STREV was the number of changes in steering wheel direction per minute); 12 Driving high frequency steering (HFS) is a proportion of high frequency steering over all steering activity expressed in the frequency domain.

Figure Titles & Legends

Figure 1 title : Immediate verbal recall (VVLT) averaged across trials A1-A3 for each condition and study phase (mean +/- se)

Figure 1 legend: The Condition*Study Phase interaction for immediate verbal recall was significant ($F[1,22]=5.58$, $p<0.05$) . Post hoc tests revealed recall was better when the placebo was consumed at arm 2 relative to arm 1 ($p<0.05$), indicating an enduring effect of CGJ after cessation of treatment. Placebo arm 1 n=15, CGJ arm 1 n=14, placebo arm 2 n=10, CGJ arm 2 n=9.

Figure 2 title: Tower of Hanoi completion time (seconds) for each condition and study phase (mean +/- se)

Fig 2 legend: The Condition*Study Phase interaction was significant ($F[1,21]=14.12$, $p<0.01$). Post hoc tests revealed completion time was significantly faster for the CGJ (n=14) relative to the placebo at arm 1 (n=15) ($p<0.01$). This difference was not significant at arm 2. Placebo arm 1 n=15, CGJ arm 1 n=14, placebo arm 2 n=10, CGJ arm 2 n=9

Figure 3 title: Steering reversals on the driving performance task for each condition and study phase (mean +/- se)

Fig 3 legend: The Condition*Study Phase interaction was significant for steering reversals ($F[1,10]=16.73$, $p<0.01$) such that during arm 1 performance was better (fewer reversals) for the CGJ condition (n=8), whereas at arm 2 performance was better for the placebo condition (n=5). However, post hoc tests did not reach significance. Placebo arm 1 n=8, CGJ arm 1 n=8, placebo arm 2 n=5, CGJ arm 2 n=5).