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Consumption of cocoa flavanols results in acute improvements in mood and cognitive performance during sustained mental effort

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Abstract

Cocoa flavanols (CF) positively influence physiological processes in ways which suggest that their consumption may improve aspects of cognitive function. This study investigated the acute cognitive and subjective effects of CF consumption during sustained mental demand. In this randomized, controlled, double-blinded, balanced, three period crossover trial 30 healthy adults consumed drinks containing 520 mg, 994 mg CF and a matched control, with a 3-day washout between drinks. Assessments included the state anxiety inventory and repeated 10-min cycles of a Cognitive Demand Battery comprising of two serial subtraction tasks (Serial Threes and Serial Sevens), a Rapid Visual Information Processing (RVIP) task and a 'mental fatigue' scale, over the course of 1 h. Consumption of both 520 mg and 994 mg CF significantly improved Serial Threes performance. The 994 mg CF beverage significantly speeded RVIP responses but also resulted in more errors during Serial Sevens. Increases in self-reported 'mental fatigue' were significantly attenuated by the consumption of the 520 mg CF beverage only. This is the first report of acute cognitive improvements following CF consumption in healthy adults. While the mechanisms underlying the effects are unknown they may be related to known effects of CF on endothelial function and blood flow.

Keywords

cocoa, cognition, cognition enhancement, flavanol, mood

Introduction

Flavonoids are a diverse class of natural compounds, ubiquitous in plants. Within the human diet, several subcategories of flavonoids predominate, including flavanols, flavonols, isoflavones, flavones, flavanones, and anthocyanidins. Among the dietary flavonoids, high levels of flavanols are found in numerous common food stuffs such as grapes, red wine, apples, both green and black teas, and cocoa and cocoa-containing products (Gu et al., 2004). They are particularly abundant in cocoa where the number and arrangement of flavanols is distinct, containing both the simple monomeric flavanols (primarily (–)-epicatechin and to a much lesser extent, (+)-catechin) as well as the structurally related dimeric and oligomeric flavanols known as procyanidins (Lazarus et al., 1999).

Over the past decade, there has been increasing interest in the potential health benefits associated with the consumption of flavanol-containing foods, some of which are likely to have ramifications for cognitive function. Dietary intervention trials have shown that the consumption of flavanol-containing cocoa products can reduce platelet aggregation (Holt et al., 2002) and improve insulin sensitivity (Grassi et al., 2005), and blood pressure (Taubert et al., 2007).There is also increasing evidence that consumption of cocoa flavanols (CF) can improve a host of parameters reflecting improved peripheral and central blood flow (Engler et al., 2004; Heiss et al., 2005, 2007; Schroeter et al., 2006; Flammer et al., 2007). While the mechanisms underlying these effects remain to be elucidated, they may be related to increased nitric oxide synthesis within blood vessels (Fisher et al., 2003; Heiss et al., 2003; Balzer et al., 2008). There is also evidence of subchronic and acute increases in blood flow following ingestion of cocoa (Balzer et al., 2008; Davison et al., 2008; Faridi et al., 2008). Of particular relevance to the current study is the finding that CF ingestion is associated with increased cerebral blood flow and brain activation. Francis et al (2006) examined the effects of CF administration on brain activation during a cognitive task. Subjects received 150 mg/day CF or a control drink for 5 days. On day 5 they underwent cognitive testing and functional magnetic resonance imaging (fMRI).

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Compared with the control, the CF condition was associated with greater activation of task-relevant brain loci (dorsolateral prefrontal cortex, anterior cingulate and parietal cortex). As the participants had received CF on the assessment day (day 5 of the subchronic trial), the possibility of acute effects on cerebral metabolism could not be precluded. The authors therefore examined the acute effects of CF on cerebral blood flow (CBF), measured using arterial spin labelling MRI, in a pilot study with four volunteers. CF ingestion resulted in increased CBF which was apparent by the first (2 h) time point and returned to baseline by the final 6 h measure. Despite the increased CBF and cortical activation, there was no concomitant improvement in task performance in the first part of the Francis study. The study used an executive/attentional switching task with relatively heavy task demands. However, prior to the intervention phase, participants were trained to a high performance criterion (greater than 95% accuracy) therefore it is possible that performance was approaching ceiling minimising the possibility of enhancement through CF ingestion.

More recently Crews et al. (2008) evaluated the cognitive effects of cocoa. Over-60-year-olds were administered 37 g dark chocolate (397 mg cocoa procyanidins) and a 273-ml cocoa drink (total 357 mg cocoa procyanidins) or matching placebo products daily for 6 weeks. There were no treatment-related changes in cognitive function, or in numerous physiological or other biomarkers measured during mid-point or end-point assessments. While these findings might suggest no efficacy for CF (procyanidins) in the cognitive arena, the authors of that study themselves acknowledge that the lack of effects may be due to either a flavonoid-rich habitual diet or the fact that these were cognitively high functioning individuals. Pulse rate was significantly elevated at weeks 3 and 6 in the cocoa group suggesting that the treatment was having some physiological effect over and above diet though the authors suggest that this may be attributable to the methylxanthines (caffeine and theobromine) present in cocoa products. Again the possibility that participants were approaching ceiling performance, thereby minimising any CF effects on cognition, cannot be ruled out.

One way of effectively eliminating ceiling effects is to subject participants to sustained effortful processing which leads to a progressive performance decline even in healthy young adults. This approach has proved to be a sensitive means of capturing the positive cognitive effects of a number of dietary interventions. The Cognitive Demand Battery (CDB) consists of repeated cycles of Serial Threes, Serial Sevens, the Bakan Rapid Visual Information Processing task and self-rated 'mental fatigue.' Over six repetitions mental fatigue ratings are reliably increased while performance declines. A series of double-blinded, placebo-controlled trials has revealed that aspects of this decline can be protected to some degree by administration of caffeine-glucose drinks (Kennedy and Scholey, 2004), by ginseng (Reay et al., 2005, 2006), glucose (Reay et al., 2006) and by a multivitamin-guaraná combination (Kennedy et al., 2008).

While the mechanisms underlying these effects are unknown, increasing the provision of metabolic substrates can result in cognition enhancement. Thus inhalation of pure oxygen (e.g. Moss et al., 1998; Scholey et al., 1999) or imbibing glucose (e.g. Benton et al., 1994) can enhance cognitive performance. It also appears that such effects are more marked during cognitive processing involving a relatively high level of mental effort (Kennedy and Scholey, 2000; Scholey et al., 2001, 2003, 2008). In addition, studies that have shown improved cognitive performance following the consumption of plant extracts such as *Panax ginseng*, *Ginkgo biloba* which may be related to their ability to enhance cerebral blood flow, oxygen utilization or glucose metabolism (Scholey and Kennedy, 2002; Reay et al., 2005, 2006). Given their effects on vascular function and blood flow, we hypothesized that the consumption of CF should positively modulate performance on mentally demanding tasks and ameliorate the increase in mental fatigue associated with performing such tasks.

The current controlled, randomized, double-blinded, balanced cross-over study therefore examined the acute cognitive and mood effects of differing levels of CF in a cocoa drink. The effects of consuming 520 mg and 994 mg CF on these parameters were compared to those of a nutrient-matched, low flavanol control drink.

Materials and methods

Design

This was a double-blinded, placebo-controlled, three period crossover study. On different visits participants underwent the Cognitive Demand Battery following consumption of a low CF control drink and drinks containing 520 mg and 994 mg of CF.

Participants

Thirty healthy non-smoking undergraduate and postgraduate student volunteers (13 male) were each paid £80 to participate in the study. The cohort had a mean age of 21.9 years (range 18–35, ± SEM 0.61 years) and mean BMI of 23.0 (± 0.62 kg/m^2). They were informed that they would be taking part in a psychological study investigating the effects of cocoa components on mental function. All participants reported that they were in good health and free from illicit drugs and over-the-counter or prescription medication (with the exception of the contraceptive pill). Participants abstained from chocolate/cocoa consumption, caffeine and alcohol consumption for a minimum of 12 h prior to the first testing session, and throughout the morning until the final testing session was completed. A diary was provided to allow participants to record all food and drink consumption for 24 h prior to the first test session of each study day. It was recommended to participants that they should avoid food and beverages which are high in flavonoid content for 24 h preceding each study day. A list of such products was provided to the participants (it was stressed that this was merely a recommendation and honesty in filling out the diary was more important). This request for restricted flavonoid diet was intended to allow acute changes in plasma flavanol levels to be monitored. The study was approved by the Northumbria University Division of Psychology Ethics Committee, and was conducted in accordance with the Declaration of Helsinki.

Salivary caffeine levels

Saliva samples were obtained using salivettes (Sarstedt, Leicester, UK). Samples were taken immediately following baseline assessment in order to confirm compliance to overnight abstinence, and following the post-treatment assessment session to confirm uniform caffeine absorption across conditions. The saliva samples were immediately frozen at −20°C until thawing for in-house batch analysis using the Emit system (Syva, Palo Alto, USA). This is an enzyme immunoassay intended to measure caffeine as a metabolite and is based on competition for antibody binding sites between caffeine and an enzyme labelled drug.

State-Trait Anxiety Inventory (STAI) state scale

The STAI state scale (Speilberger et al., 1969) was administered as a self-completed questionnaire. The scale contains 20 four-point items which record the presence (e.g. 'I am tense') and absence (e.g. 'I feel at ease') of anxiety symptoms. These are combined to provide a sum score between 20 and 80 (a lower score representing lower anxiety). The scale is sensitive to laboratory stressors and certain dietary manipulations (Kennedy et al., 2005).

Cognitive Demand Battery (CDB)

The Cognitive Demand Battery comprised of two computerized serial subtraction tasks (Serial Threes and Serial Sevens) the Bakan Rapid Visual Information Processing task (RVIP) and a paper-and-pencil mental fatigue scale implemented as follows:

(1) Serial Threes subtraction task: Participants were required to count backwards in threes from a given number as quickly and as accurately as possible using the computer keyboard number keys to enter each response. A random starting number between 800 and 999 was presented on the computer screen, which was cleared by the entry of the first response. The task was scored for number of correct responses and number of errors. In the case of incorrect responses, subsequent responses were scored as positive if they were scored as correct in relation to the new number for details see Scholey et al. (2001). The duration of this task was 2 min.
(2) Serial Sevens subtraction task: This was identical to the Serial Threes task with the exception that it involved serial subtraction of sevens. Again the duration was 2 min.
(3) RVIP task: The participant was required to monitor a continuous series of digits for targets of three consecutive odd or three consecutive even digits. The digits were presented at the rate of 100 per minute and the participant responded to the detection of a target string by pressing the 'space bar' as quickly as possible. The task was continuous, with eight correct target strings being presented in each minute. The task was scored for percentage of target strings correctly detected, average reaction time for correct detections, and number of false alarms. The task lasted for 5 min.
(4) 'Mental fatigue' visual analogue scale: Participants rated their current subjective mental fatigue state by making a mark on a 100 mm Visual Analogue Scale (VAS) with the end points labelled 'not at all'(left hand end) and 'very much so'(right hand end). One minute was allowed to complete the VAS.

The total duration of each cycle of the CDB (Serial Threes, Serial Sevens, RVIP, 'mental fatigue' VAS) was 10 min.

Flavanol-containing test drinks

On each day of testing participants consumed one of three dairy-based cocoa drinks containing a total of either: 1) 46 mg CF ('control'); 2) 520 mg cocoa flavanols ('520 CF'), or 3) 994 mg cocoa flavanols ('994 CF'). The lower level coincides with the CF drink which produced increased brain–blood flow at 2 h (Francis et al., 2006). Total cocoa flavanol content is defined here as the sum of all monomeric flavanols and their structurally related dimeric and oligomeric procyanidins up to decamer. The total cocoa flavanol content was determined by Mars Inc according to published methodology (Robbins et al., 2008).

Test products were manufactured and supplied by Mars (Leicestershire, UK) as a dry cocoa blend in individually wrapped sachets, coded with a three-digit random number. These were stored in a cool, dry environment prior to drink preparation. The sachets contained either 23 mg cocoa flavanols/sachet ('low flavanol' cocoa drink mix) or 497 mg CF/sachet ('high flavanol' cocoa drink mix). The high flavanol cocoa drink mixes were prepared using a Cocoapro® processed cocoa powder, To ensure that the macronutrients, micronutrients, and alkaloids (caffeine and theobromine) were matched in all conditions of the study, two sachets of the beverage mixes were combined to produce the control drink (2× 'low flavanol' sachets, 46 mg CF); the 520 mg CF drink (1× 'low flavanol,' 1× 'high flavanol' sachet), or the 994 mg CF drink (2× 'high flavanol' sachets). Details of the nutritional information for the preparations can be found in Table 1. On the day of testing, coded drinks were prepared by mixing the two sachets with 200 ml hot water by a disinterested third party staff member who was not involved in the study and who played no further part in the experiment. The study participants were asked to consume the drink within 5 min. The general composition of the drinks was as follows: non-fat dry milk powder (59.0%); cocoa powder (33%), fibre (5.6%); emulsifier (0.8%); cellulose gel (0.55%); xanthan gum (0.55%); artificial and natural vanilla (0.1%); and sucralose (0.1%).

Procedure

Prior to participation in the study, volunteers signed an informed consent form and completed a medical health questionnaire. Each participant was required to attend a total of four study days (one practice visit and three study visits) that were conducted not less than three days apart to ensure sufficient wash out between conditions. They were asked to consume the same light breakfast on study days (this was checked using the food diaries). Testing took place in a suite of dedicated university laboratories with participants visually isolated from each other. On arrival at their first

Table 1. Nutritional content of the three study beverages

	low CF control drink	'520 CF' drink	'994 CF' drink
Total cocoa flavanols, mg, comprising:	46	520	994
Epicatechin	4	94	184
Catechin	8	35	62
Dimers	10	96	182
Trimers-decamers	24	295	566
Calories	216	217	218
Total fat, g	2.6	2.6	2.6
Sat. fat, g	1.4	1.4	1.4
Cholesterol, mg	9.8	9.5	9.2
Total carbohydrate, g	30.8	31.2	31.6
Dietary fibre, g	7.4	6.5	5.6
Sugars, g	17.2	17.4	17.6
Protein, g	17	17	17
Caffeine, mg	46.4	43.5	40.6
Theobromine, mg	400.2	429.2	458.2
Sodium, mg	450	415.2	380.4
Potassium, mg	1194.8	1087.5	980.2
Calcium, mg	451.2	454.7	458.2
Iron, mg	8.6	6.6	4.6
Phosphorus, mg	495.6	508.5	521.4
Magnesium, mg	141	149.4	157.8
Zinc, mg	3	2.9	2.8
Copper, mg	0.8	0.7	0.6
Manganese, mg	1	1.1	1.2

session on the first day, participants were randomly allocated to one of six treatment orders of the Latin square design that counterbalanced the order of treatments across the three study days of the study. Prior to these a practice visit involved completion of the test battery six times. This was undertaken in order to control for practice effects and to allow familiarization with the test battery and procedure on subsequent visits. The practice day data were not included in any analyses.

The structure of each of the three active study days is summarized in Figure 1. Following arrival at the laboratory and a short assimilation period, participants provided a 2 ml venous blood sample for determination of serum flavonoid levels, filled out the state portion of the Spielberger state-trait anxiety inventory, and completed an initial practice run through the 10 min Cognitive Demand Battery. This consisted of Serial Threes (2 min), Serial Sevens (2 min), RVIP (5 min), and the 'mental fatigue' VAS (1 min). The computerized tests were administered on Dell PCs running Windows. Participants completed a pre-dose 10-min Cognitive Demand Battery in order to establish that day's baseline performance. They then provided a pre-treatment saliva sample using a salivette for determination of caffeine levels (this was taken at the last opportunity prior to CF consumption to maximise the detection of caffeine from products consumed on the morning of study days). This was followed immediately by ingestion of that day's drink (five minutes was allowed for consumption). Starting a fixed 90 min following beverage consumption (during which participants were allowed to leave the laboratory but were instructed to consume nothing other than water and to avoid any strenuous exercise), participants underwent the Cognitive Demand Battery a further six times in succession (i.e. for 60 min in total). They then provided a second, final saliva sample. Study visits were repeated at the same time of day for each participant, starting at 0815 and 0930, until all treatments had been completed.

Data treatment and statistics

The primary outcomes were the performance and fatigue scores during the Cognitive Demand Battery (CDB). There were eight CDB measures which were analysed as change-from-baseline scores: number of errors and number of correct responses from both Serial Subtractions tasks; % accuracy, reaction times and false alarms from the RVIP, and mental fatigue ratings. There were two measure each for state anxiety (on arriving at the laboratory and immediately following the CDB) and salivary caffeine (immediately prior to drink consumption and immediately after the CDB).

To test for chance baseline differences which may have skewed change-from-baseline scores, prior to the primary statistical analysis, all pre-dose baseline measures were subjected to one-way repeated measures ANOVAs. In the case of salivary caffeine, baseline levels were also examined to assess compliance to caffeine abstinence instructions. Additionally to ascertain any interactions with treatment order, initial three-way (treatment order $\times$ treatment $\times$ time) ANOVAs were conducted incorporating the six treatment orders from the Latin square.

The primary analyses (where there was no interaction with, or main effect of treatment order) involved two-way (treatment $\times$ time) repeated measures ANOVAs on 'change-from-baseline' data from each CDB measure. At each post-treatment time point, the score from the flavanol-poor control was compared to that for each of the two active treatments using planned comparisons, utilising *t*-tests with MSError from an omnibus ANOVA as an error term (Keppel, 1991). All testing was two-tailed, comparisons were strictly planned prior to the study, were restricted to the number of conditions minus one at each time point, and only probabilities associated with these pre-planned comparisons were calculated.

All analyses were performed using SPSS v15 for Windows.

Results

Baseline scores

There were no statistically significant baseline differences for any measure.

Treatment order

For state anxiety there was a significant main effect of treatment order [$F(5.24) = 2.667$, $p = 0.047$]. There were no other main effects nor interactions with treatment order, therefore results of treatment $\times$t time ANOVAs are reported.

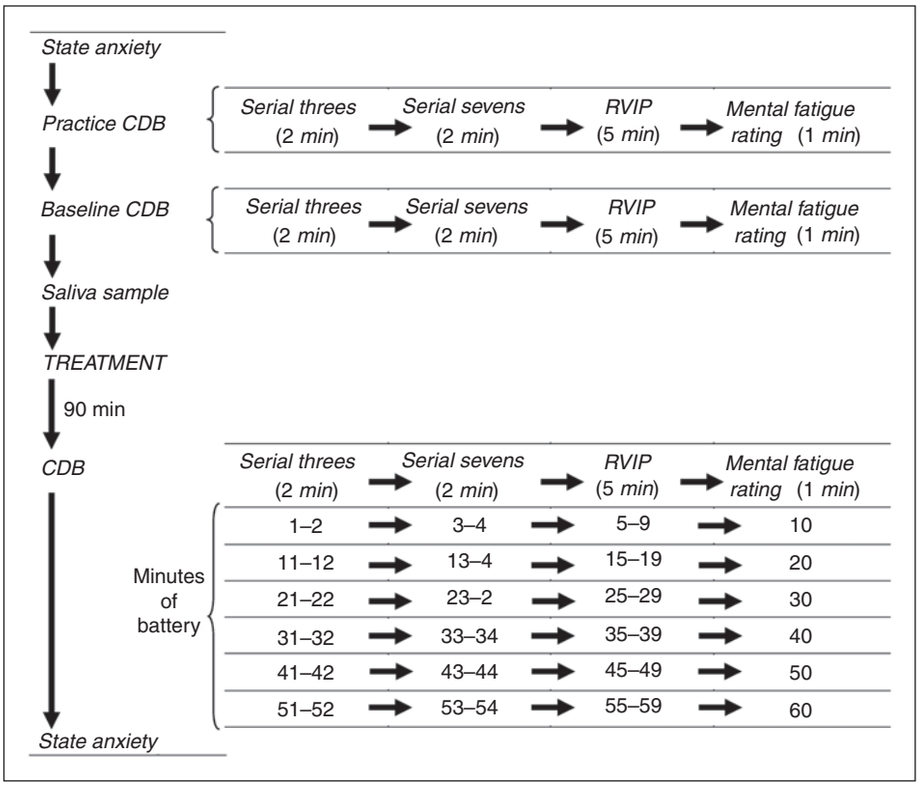

Figure 1. Structure of study days. An initial 10-min Cognitive Demand Battery (CDB) was followed by the day's baseline CDB. A saliva sample was taken for determination of caffeine prior to the day's treatment. 90 min was allowed for absorption of CF followed by six cycles of the 10-min CDB (with minutes of battery shown in relation to tasks). State anxiety was assessed at the beginning and end of the procedure.

Salivary caffeine levels

Salivary analysis confirmed adherence to caffeine abstinence instructions with mean baseline caffeine values of 0.35 μg/ml (levels just below 1 μg/ml have been reported for overnight caffeine abstinence (Evans and Griffiths, 1999). There was a significant main effect of time [$F(1.46) = 24.533$, $p < 0.001$] with higher caffeine levels at the second, final reading than pre-treatment, confirming that the three drinks were matched for caffeine content (see Table 1). There was no significant main effect of treatment, nor any treatment × time interaction.

State anxiety

There were no significant effects on the Spielberger state anxiety questionnaire ($F < 1$ in all cases), but see 'Treatment order' above.

Cognitive demand battery

Mean pre-dose baseline, and change from baseline scores for each intervention are presented in Table 2, along with *F*-values and probabilities associated with treatment effects. Change from baseline scores for each variable are also plotted in Figure 2.

Serial Three subtractions: Compared with the control drink, the number of correct serial threes was significantly increased following consumption of drinks containing both the 520 and 994 mg CF (Figure 2a). There were a higher number of correct responses following the consumption of 520 mg CF at all time points of the battery (10 min [$t(290) = 2.91$, $p = 0.004$], 20 min [$t(290) = 3.48$, $p = 0.0006$], 30 min [$t(290) = 3.07$, $p = 0.002$], 40 min [$t(290) = 4.51$, $p < 0.00001$], 50 min [$t(290) = 3.62$, $p = 0.0004$], and 60 min [$t(290) = 2.56$, $p = 0.01$]). Following consumption of 994 mg CF, the number of correct responses was significantly increased at 10 min [$t(290) = 2.26$, $p = 0.02$], 20 min [$t(290) = 2.80$, $p = 0.005$], 30 min [$t(290) = 2.26$, $p = 0.02$], and 40 min [$t(290) = 3.83$, $p = 0.0002$]. There were a single statistically significant effects of drink condition on number of Serial Three errors (Figure 2b), with fewer errors at 20 min following 994 mg CF drink compared with the control [$t\ (290) = 1.98$, $p = 0.048$]

Serial Seven subtractions: There were no statistically significant effects associated with either active drink on the number of Serial Sevens completed (see Figure 2c). Consumption of the 994 mg CF drink was associated with significantly more errors at 30 min [$t(290) = 2.31$, $p = 0.02$] and 40 min [$t(290) = 5.19$, $p < 0.00001$], see Figure 2d.

RVIP: There were no significant treatment effects on RVIP accuracy (see Figure 2e). Following the 994 mg CF drink,

Table 2.

Measure	Treatment	Pre-dose baseline score	Post-dose change from baseline score						F	p
			10 min	20 min	30 min	40 min	50 min	60 min		
Serial Threes correct (number)	Control	44.3 ± 2.83	0.43 ± 1.22	−0.37 ± 1.21	0.43 ± 1.29	−0.50 ± 1.54	−0.70 ± 1.68	1.93 ± 1.45	18.6	<0.001
	520 mg CP	42.8 ± 2.32	4.00 ± 1.09	3.90 ± 1.00	4.20 ± 1.51	5.03 ± 1.66	3.73 ± 1.54	5.07 ± 1.37		
	994 mg CP	43.0 ± 2.29	3.20 ± 1.13	3.07 ± 1.26	3.20 ± 1.24	4.20 ± 1.02	1.60 ± 1.75	3.13 ± 1.12		
Serial Threes errors (number)	Control	2.90 ± 0.45	0.20 ± 0.42	0.70 ± 0.39	0.43 ± 0.46	0.13 ± 0.41	1.03 ± 0.45	0.60 ± 0.50	4.67	0.01
	520 mg CP	3.00 ± 0.34	−0.30 ± 0.28	0.63 ± 0.39	0.03 ± 0.42	0.43 ± 0.43	0.47 ± 0.54	0.53 ± 0.39		
	994 mg CP	3.67 ± 0.41	−0.43 ± 0.48	−0.37 ± 0.57	−0.17 ± 0.39	−0.67 ± 0.56	0.43 ± 0.67	−0.30 ± 0.48		
Serial Sevens correct (number)	Control	25.3 ± 2.10	2.87 ± 1.11	2.80 ± 0.97	2.70 ± 1.16	3.80 ± 1.06	3.67 ± 1.00	3.77 ± 1.17	<1	–
	520 mg CP	26.5 ± 2.07	1.50 ± 0.92	2.40 ± 0.98	2.07 ± 0.97	3.57 ± 0.94	3.83 ± 0.93	3.53 ± 0.95		
	994 mg CP	26.6 ± 1.88	1.63 ± 1.01	2.43 ± 0.97	2.57 ± 1.19	2.07 ± 1.28	2.80 ± 1.22	3.67 ± 1.14		
Serial Sevens errors (number)	Control	3.43 ± 0.37	−0.43 ± 0.43	−0.10 ± 0.51	−0.13 ± 0. 54	−0.57 ± 0.43	0.93 ± 0.53	0.97 ± 0.57	4.12	0.02
	520 mg CP	3.13 ± 0.42	−0.17 ± 0.38	−0.07 ± 0.30	0.53 ± 0.31	−0.27 ± 0.38	0.20 ± 0.36	0.83 ± 0.38		
	994 mg CP	3.03 ± 0.37	0.37 ± 0.43	0.17 ± 0.35	0.93 ± 0.51	1.83 ± 0.59	0.83 ± 0.53	0.43 ± 0.48		
RVIP accuracy (%)	Control	63.8 ± 3.57	3.43 ± 2.09	3.24 ± 2.38	−0.46 ± 2.49	−0.83 ± 2.33	0.37 ± 2.17	−0.46 ± 2.36	1.13	>0.1
	520 mg CP	65.2 ± 3.65	6.48 ± 1.85	1.76 ± 2.09	1.94 ± 2.31	−2.41 ± 2.51	−2.22 ± 1.85	1.39 ± 2.31		
	994 mg CP	65.2 ± 3.11	6.11 ± 2.58	3.89 ± 2.32	3.43 ± 3.46	0.65 ± 2.38	0.93 ± 2.92	0.56 ± 2.44		
RVIP reaction time (msec)	Control	479 ± 11.4	−8.29 ± 8.94	−9.23 ± 7.77	4.28 ± 7.86	11.5 ± 11.0	3.22 ± 7.26	−11.1 ± 8.47	3.66	0.03
	520 mg CP	476 ± 10.4	−15.9 ± 6.17	−5.25 ± 6.76	−4.88 ± 6.67	1.05 ± 6.83	−2.98 ± 6.97	−7.22 ± 6.11		
	994 mg CP	482 ± 11.8	−18.3 ± 7.13	−14.2 ± 8.73	−11.9 ± 9.04	−6.66 ± 8.07	−1.57 ± 9.66	−20.2 ± 9.52		
RVIP false alarms (number)	Control	1.44 ± 0.48	−0.15 ± 0.32	0.11 ± 0.31	0.59 ± 0.34	0.37 ± 0.53	0.26 ± 0.51	0.59 ± 0.58	2.97	0.05
	520 mg CP	1.59 ± 0.37	−0.07 ± 0.37	0.22 ± 0.37	−0.19 ± 0.33	0.26 ± 0.43	−0.11 ± 0.31	0.04 ± 0.26		
	994 mg CP	1.11 ± 0.25	0.22 ± 0.26	0.37 ± 0.34	0.56 ± 0.34	0.81 ± 0.37	0.59 ± 0.33	0.56 ± 0.41		
'Mental fatigue' VAS (mm)	Control	25.0 ± 3.70	−6.34 ± 2.23	−1.52 ± 2.42	4.03 ± 2.84	12.0 ± 2.80	17.1 ± 2.90	23.3 ± 3.37	0.790	>0.1
	520 mg CP	25.4 ± 4.44	−11.7 ± 2.87	−6.69 ± 2.80	0.69 ± 3.89	6.59 ± 3.90	11.8 ± 4.17	16.8 ± 4.56		
	994 mg CP	22.7 ± 4.15	−7.28 ± 3.13	−1.97 ± 3.22	4.66 ± 3.61	10.6 ± 3.89	16.6 ± 4.17	21.5 ± 4.45		

Mean (±SEM) baseline and change-from-baseline scores for each outcome. F- and p-values associated with treatment effects are also presented. RVIP, Rapid Visual Information Processing; VAS, Visual Analogue Scale.

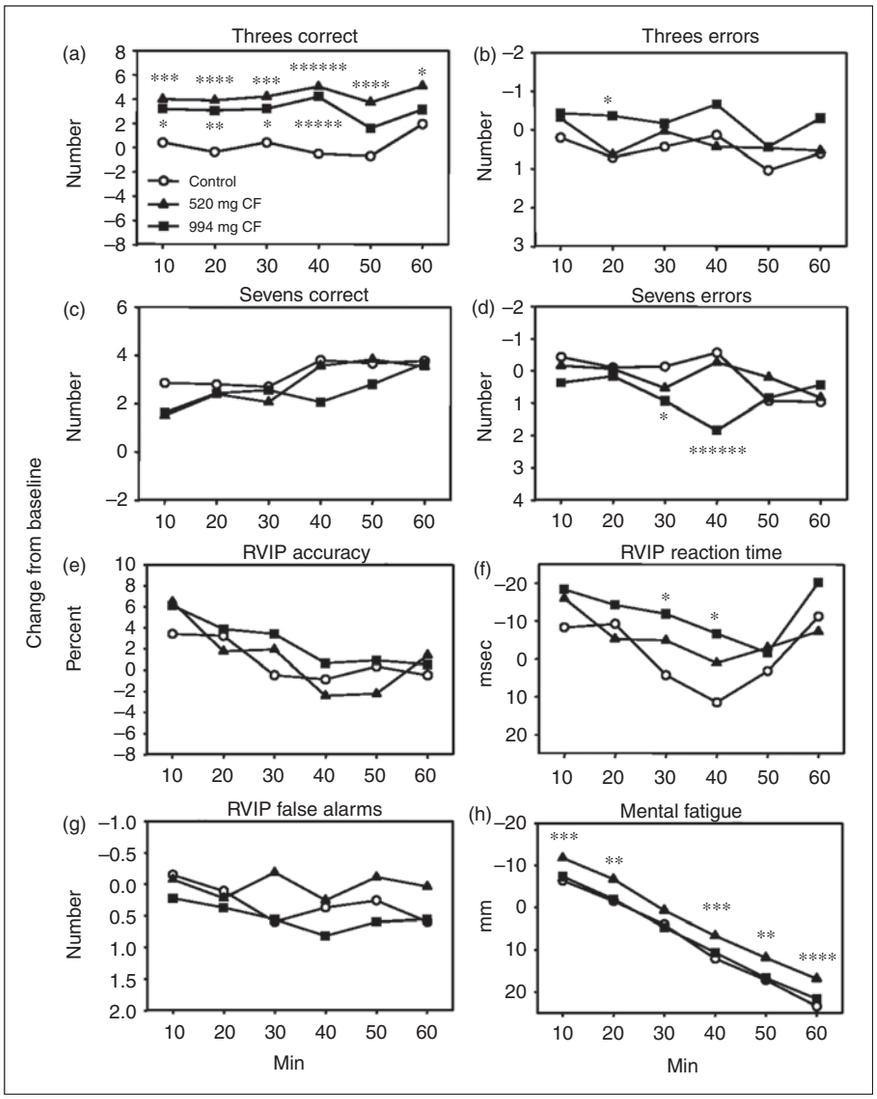

Figure 2. Mean change from baseline scores following control, 520 mg CF and 994 mg CF drinks. Significant differences compared with placebo are indicated (*$p < 0.05$, **$p < 0.01$, ***$p < 0.005$, ****$p < 0.001$, *****$p < 0.0005$, ******$p < 0.0001$). Please note all graphs are plotted such that higher values on the y-axis represent better performance or mood.

participants were significantly faster on the RVIP task at 30 min [$t(260) = 2.27$, $p = 0.02$] and 40 min [$t(260) = 2.55$, $p = 0.01$], see Figure 2f. There were no significant treatment effects on the number of false alarms during this task (see Figure 2g).

Mental fatigue VAS: Following the 520 mg CF drink, self-rated mental fatigue was significantly improved at all but the 30 min time point (10 mins [$t(280) = 2.85$, $p = .0047$], 20 min [$t(280) = 2.76$, $p = 0.006$], 40 min [$t(280) = 2.89$, $p = .004$], 50 min [$t(280) = 2.83$, $p = 0.005$], and 60 min [$t(280) = 3.48$, $p = 0.0006$]), see Figure 2h.

Discussion

The results of the current study demonstrate, for the first time, that the acute consumption of CF can improve performance and reduce 'mental fatigue' during highly effortful cognitive processing in healthy young participants. Compared with the nutrient-matched, low flavanol control drink, improvements to cognition and mood were evident following consumption of both 520 mg CF and 994 mg CF. Of these, the 520 mg CF drink appeared to be more beneficial. Both CF-rich drinks positively influenced the same performance measures, but there was also some 'cost' to performance following the 994 mg CF drink. Moreover the benefits associated with the 520 mg CF drink were apparent at a greater number of time points and included reduced mental fatigue ratings. The test drinks were matched for other nutritional and alkaloid content, thus we can be reasonably confident that these effects are attributable to CF rather than other components in the product.

Consumption of 520 mg CF reliably improved Serial Threes performance. These improvements were demonstrated

at each post-dose completion of the battery with the most striking improvement during the fourth CDB cycle (130 min post-CF). Improved Serial Threes performance was also observed for the 994 mg CF intervention. However, this effect was less striking and apparent only for the first four cycles of the battery (again with the most significant improvement during the fourth cycle). Consumption of 994 mg CF led to faster RVIP during the third and fourth completion, an effect not seen following the 520 mg CF. The only negative effect was also associated with 994 mg CF, with more Serial Sevens errors during the third and fourth cycle.

The differential effects to Serial Threes and Serial Sevens in the current study has been observed previously. Utilising the same Cognitive Demand battery as here, (Reay et al., 2005) found significant improvements to Serial Sevens but no interpretable effect on Serial Threes following 200 mg *Panax ginseng*. Conversely, in a follow-up study, Reay et al. (2006) found the opposite pattern. Kennedy and Scholey (2004) also found improvements to Serial Sevens following consumption of 68 g glucose plus 46 mg caffeine with no effects on Serial Threes. These data suggests that the two tasks may draw on different neurocognitive substrates. Both tasks have attentional components, but Serial Threes may be more limited by psychomotor performance, whereas the Serial Sevens task relies more heavily on working memory and executive function. These results again demonstrate the utility of the battery in capturing acute effects of nutritional interventions.

Turning to the subjective outcomes, following the consumption of 520 mg CF, there was an attenuation of the characteristic increase in 'mental fatigue' seen with successive completions of this battery. Mental fatigue ratings following consumption of 520 mg CF were significantly lower than placebo during all but the third cycle of the of the battery. There were no effects on this measure following consumption of 994 mg CF. 'State anxiety' was unaffected by any treatment.

In the current study, the greatest number and most robust mood and cognition effects were seen at the during the fourth CDB cycle, i.e.130 min post-CF ingestion. This time point more or less coincides with the 2 h post-CF peak in CBF (Francis et al., 2006) and plasma epicatechin levels (Rein et al., 2000) reported in previous studies. Our intention was to examine any relationship between plasma flavonoids and neurocognitive function, unfortunately damage to samples during shipping prevented this from happening.

Although analysis of blood flavanols was not possible, it may be that blood sampling influenced anxiety levels. Anxiety appeared not to be influenced by the CDB or by CF, however as this was the single measure which was significantly affected by treatment order we cannot draw any conclusions about anxiety from this study. It is also possible that anxiety may have been influenced by the methylxanthines contained within the CF drinks. Although these were well matched across drinks (see Table 1), there were small differences. Furthermore we cannot rule out the possibility of caffeine, theobromine or indeed other components of the drinks such as magnesium (Grassi et al., 2005a) interacting differentially with CF to produce the effects seen here.

The effects of lower levels of CF appear to merit further exploration and, if possible, the lowest psychoactive dose should be established. Of relevance here is a recent finding of impairment to performance following consumption of very low doses of caffeine (Haskell et al., 2008). However unlikely, this raises the possibility that acute negative effects may occur in response to low levels of CF, this would serve to exaggerate the effects of the two higher CF preparations in the current study. The possibility could be addressed with a partial replication of the current study with the inclusion of an additional, flavanol-free arm.

The effects of consuming 520 mg CF on Serial Threes and 'mental fatigue' ratings were apparent during the first cycle of the CDB. This raises the possibility that cognitive benefits may have been observed if the CBD had commenced earlier than 90 min post-drink. The effects seen 40 min into the battery may also represent an optimal interaction between flavanol levels and reduced resources due to the demanding and fatiguing nature of the tasks. It is also possible that effects were most pronounced at this point due to participants reaching a peak in stress levels. If so we might attribute adaptogenic (or resistance-increasing) properties to CF. This could be further explored by monitoring performance during psychological stress following CF ingestion. Although adaptogenic plants have been shown to have acute effects (Panossian and Wagner, 2005), by definition – i.e. producing adaptive adjustments – they are traditionally thought of as building resistance to stressful events through chronic rather than acute administration. It would therefore be of great interest to explore the role of CF as adaptogens by studying their chronic effect and comparing to those elicited acutely as in the current study. Certainly there is epidemiological evidence to suggest that long-term flavanol intake has neurocognitive benefits including neuroprotection (for reviews see Commenges et al., 2000; Letenneur et al., 2007; Patel et al., 2008; Spencer, 2008). On the other hand, seemingly compelling epidemiological data are often not translated into positive findings in clinical trials. Nevertheless future studies might usefully collect data regarding include habitual consumption of methylxanthine, CF and other drink components which may have interacted with fluctuations in acute levels to influence aspects of cognitive performance.

The exact mechanisms by which CF may benefit cognitive processing remain unknown. There has been a great deal of focus on the anti-oxidant properties of CF. However this notion has recently been challenged (for reviews see Frei, 2004; Halliwell, 2008). Firstly CF undergo extensive biotransformation following ingestion rendering molecules with greatly reduced anti-oxidant capacity, and the absorbed fragments of CF do not possess the anti-oxidant properties observed in the whole molecule. Additionally it appears that there may have been over-interpretation of CF anti-oxidant capacity ex vivo. Thus it seems that the anti-oxidant properties of CF may have been overstated and this role remains to be substantiated in physiological systems using realistic levels of orally administered CF.

Previous studies have shown that the acute consumption of 200–500 mg of CF is able to increase blood vessel dilation (Heiss et al., 2003, 2005, 2007; Engler et al., 2004). We have previously hypothesized that the delivery of metabolic substrates contributes to enhanced performance of cognitively demanding tasks (Scholey, 2001; Scholey et al., 2001, 2006, 2008;

Scholey and Kennedy, 2004). If true then it seems plausible that such effects may underlie the cognitive benefits observed here. Indeed the fact that peak behavioural effects were observed at around two hours post-consumption is consistent with peak plasma epicatechin levels (Rein et al., 2000), cerebral blood flow (Francis et al., 2006) and dose-dependent increases in flow-mediated dilatation (FMD) peaking at the same time point (Schroeter et al., 2006). Similar observations have been made in diabetic patients (Balzer et al., 2008), healthy overweight individuals (Faridi et al., 2008) and healthy smokers (Hermann et al., 2006). These results support previous findings which have shown that natural products which increase cerebral blood flow and/or metabolic activity are particularly effective in improving cognitive processing during prolonged mental effort. On the other hand it should also be noted that a recent study found no acute effect of 450 mg CF on cerebral blood flow (CBF) in healthy elderly individuals (Sorond et al., 2008). The study reported increased mean flow velocity (MFV) in the middle cerebral artery measured by Doppler ultrasound after one and two weeks CF supplementation. The effects reached significance 8 h following CF treatment on days 7 and 14. However there were no significant effects on this measure on day 1, indeed there was a slight (non-significant) fall in MFV 2–4 h post CF consumption. The difference in these findings and those reporting acute increases to measures of vaso-activity most likely represent differences in specific measures used.

In conclusion we believe that this is the first report of acute cognitive benefits to cognitive function associated with CF consumption in healthy volunteers. These findings suggest that the consumption of 520 mg CF (and to a lesser extent 994 mg CF) may be beneficial to performance and mood during highly effortful cognitive processing. These effects merit further exploration in other populations, over longer time scales, at different doses, while co-monitoring relevant biomarkers (including neuroimaging) and in other cognitive domains.

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