Epigallocatechin gallate (EGCG), cerebral blood flow parameters, cognitive performance and mood in healthy humans: a double-blind, placebo-controlled, crossover investigation.

Journal:	*Human Psychopharmacology: Clinical and Experimental*
Manuscript ID:	HUP-11-0091.R2
Wiley - Manuscript type:	Research Article
Date Submitted by the Author:	n/a
Complete List of Authors:	WIGHTMAN, EMMA; NORTHUMBRIA UNIVERSITY Haskell, Crystal; Northumbria University, Psychology Forster, Joanne; Northumbria University, Psychology Veasey, Rachel; Northumbria University, Psychology Kennedy, David; Northumbria University, Psychology
Keyword:	EGCG, Epigallocatechin gallate, Cerebral blood flow, Cognitive, Mood, NIRS

Epigallocatechin gallate (EGCG), cerebral blood flow parameters, cognitive performance and mood in healthy humans: a double-blind, placebo-controlled, crossover investigation.

Emma. L. Wightman[1], Crystal. F. Haskell[1], Joanne. S. Forster[1], Rachel. C. Veasey[1], David. O. Kennedy[1].

[1]Brain, Performance and Nutrition Research Centre, Northumbria University, Newcastle upon Tyne, United Kingdom, NE1 8ST.

Author for correspondence:

David. O. Kennedy

Brain, Performance and Nutrition Research Centre

Northumbria University

Newcastle, UK

NE1 8ST

Tel: (+44)191 2437720

Fax: (+44)191 2437820

e-mail: david.kennedy@northumbria.ac.uk

Running title: EGCG and cerebral blood flow

Clinicaltrials.gov ID: NCT00981292

Key Words: EGCG; epigallocatechin gallate; cerebral blood flow; cognitive, mood.

ABSTRACT

OBJECTIVE: To assess the effects of oral ingestion of the **green tea** polyphenol epigallocatechin gallate (EGCG) on cognitive performance, mood and localised cerebral blood flow (CBF) parameters in healthy human adults.

METHOD: In this double blind, placebo controlled, cross-over study 27 healthy adults received placebo and two doses (135 mg and 270 mg) of EGCG in counterbalanced order on separate days. Following a 45-min resting absorption period participants performed a selection of computerised cognitive tasks that activate the frontal cortex for a further 42 minutes. CBF and haemodynamics, as indexed by concentration changes in oxygenated and deoxygenated haemoglobin, were assessed in the frontal cortex throughout the post-treatment period using Near Infrared Spectroscopy (NIRS).

RESULTS: During the post-dose task performance period the administration of 135 mg EGCG resulted in reduced CBF in the frontal cortex, as indexed by significantly lower concentrations of both oxygenated and total haemoglobin, in comparison to placebo. Heart rate was significantly reduced from pre- to post dose across all treatments. No significant differences were observed for the level of deoxygenated haemoglobin or on any of the cognitive performance/mood measures.

CONCLUSIONS: These results demonstrate that a single dose of orally administered EGCG can modulate CBF parameters in healthy humans **but that this is not associated with changes in cognitive performance or mood**.

INTRODUCTION

Epigallocatechin gallate (EGCG) is the most abundant polyphenol found in the tea plant (*Camellia sinensis)*, accounting for ~10% of the dry weight of green tea leaves (Mandel et al., 2008). Within the plant EGCG and other catechins confer protective and growth promoting roles, accumulating in vulnerable locations such as the seed coat, woody stem, root, and foliage (Forrest and Bendall, 1969). It has been suggested that the mechanisms by which tea catechins protect the host plant may also exert a range of protective health effects in the humans who consume **them** (Khan and Mukhtar, 2007), including possible protection against high blood cholesterol and high blood pressure (Taubert et al., 2007), and ischemic heart disease (Arts et al., 2001).

In vitro studies suggest that EGCG has a number of properties potentially relevant to brain function, including effects on cholinergic transmission (Katayama et al., 2002), neurite outgrowth (Reznichenko et al., 2005), inflammatory parameters (Kim et al., 2007), metal chelation (Mandel et al., 2008) and an ability to act as an anti-oxidant (Unno et al., 2007) and to up-regulate endogenous antioxidant capacity (Mandel et al., 2008). *In vivo* studies utilising animal models suggest that EGCG may offer multi-faceted protection against the aetiology and behavioural consequences of a number of neurodegenerative diseases **after oral administration**, including Alzheimer's disease (Koh et al., 2006; Lee et al., 2009; Rezai-Zadeh et al., 2008) and Parkinson's disease (Levites et al., 2001). **Epidemiological studies also suggest that tea consumption reduces the risk of ischemia/stroke in humans (Arab et al., 2009)** and green tea catechins (63% EGCG) have also been shown to improve cognitive

performance and concomitantly increase antioxidant capacity in normal rats **after oral ingestion** (Haque et al., 2004).

One property underlying the potential cardio-vascular effects of EGCG is an ability to modulate blood flow parameters. *Ex vivo* research in isolated rat aorta suggests that EGCG exhibits vaso-relaxant properties (Lorenz et al., 2009; Chen et al., 2000; Alvarez et al., 2006). However, vaso-constriction (Sanae et al., 2002; Shen et al., 2003) and biphasic relaxant and constricting (Alvarez et al., 2004) properties have also been observed. Both the vaso-relaxation and constriction have been attributed, in part, to opposite effects on endothelial nitric oxide (NO) production and activity (Sanae et al., 2002; Lorenz et al., 2009). With regards the effects of this in humans, a number of studies have demonstrated improved endothelial function in the periphery following tea (Shenouda and Vita, 2007; Alexopoulos et al., 2008; Nagaya et al., 2004) with similar effects seen following epicatechin administered to healthy participants (Schroeter et al., 2006) and EGCG administered to patients with coronary artery disease (Widlansky et al., 2007).

Whilst the balance of evidence suggests that EGCG promotes endothelial NO synthesis, and therefore peripheral vaso-relaxation, it has also been shown to directly inhibit both inducible NO synthase (iNOS) and neuronal NO synthase (nNOS) (Chan et al., 1997; Stevens et al., 2002). While the effect of reducing neuronal NO production may confer neuroprotective properties in the face of damaging overproduction of NO during brain insults (Wei et al., 2004) the effects on cerebral blood flow (CBF) of EGCG related down-regulation of nNOS during normal functioning are unknown. NO itself is a key vaso-dilatory mediator in the neurovascular coupling of neuronal activity to increased blood supply in active tissue (Gally et al., 1990; Kitaura et al., 2007) and whilst both eNOS and nNOS are

found in brain tissue (Toda and Okamura, 2003) it is NO derived from nNOS which has been observed to make the greatest contribution to activity dependent vasodilation (Ayata et al., 1996; Cholet et al., 1997; Kitaura et al., 2007; Santizo et al., 2000).

Indeed, previous *in vivo* research with the red wine polyphenol resveratrol indicates that as well as up- regulating levels of eNOS (Tsai et al., 2007) resveratrol is also able to up- regulate nNOS (Hung et al., 2004), **unlike EGCG**, and as such is associated with both peripheral vasodilation (Rush et al., 2007; Rivera et al., 2009) and increased CBF in **the frontal cortex of** healthy humans, as measured by Near Infrared Spectroscopy (NIRS), during the performance of tasks that activate this brain region (Kennedy et al., 2010). **To date, a small number of pharmacological intervention studies have also used the technique to infer localized brain activity (Kanamaru et al., 2008) and CBF and oxygenation (Bönöczk et al., 2002) from changes in haemoglobin concentrations. These include studies demonstrating an increased haemodynamic response to task performance following docosahexaenoic acid (Jackson et al., In press) and both increased CBF following single doses of resveratrol (Kennedy et al., 2010) and decreased CBF following a single dose of caffeine (Kennedy and Haskell, 2011)**.

The current double-blind, placebo-controlled, balanced cross-over study therefore investigated the effects of oral doses of EGCG on CBF in the frontal cortex using NIRS during the performance of tasks that activate this brain region. Given the wide range of potential brain relevant parameters that might be modulated by EGCG, including blood flow and therefore the delivery of metabolic substrates, cognitive performance was also assessed during the selection of executive function/working memory and attention tasks. **Heart rate and blood pressure were also monitored at several time points throughout the study.**

EXPERIMENTAL METHODS

Participants

Twenty-seven healthy adults (11 males, 16 females, mean age 22 years, range 18-30, 23 right handed, 4 left handed) took part in the study. A further 5 participants that failed to satisfactorily complete the testing procedures were replaced during the running of the study and prior to un-blinding. All participants attended the laboratory having fasted from 2 hours prior to testing. Participants were required to consume a standardised diet prior to that in terms of what and when they ate on day 1. They abstained from caffeine and alcohol from 8pm the night before. All participants reported themselves to be in good health and free from social drugs, alcohol, prescription medication and herbal extracts/food supplements at each assessment. Participants who had suffered a head injury, neurological disorder or neuro-developmental disorder were excluded from participation, as were those who had any relevant food allergies or intolerances, smoked tobacco, drank excessive amounts of caffeine (more than 600mg day as assessed by a caffeine consumption questionnaire) or took illicit social drugs.

The study received ethical approval from the Northumbria University School of Psychology and Sport Sciences Ethics Committee and was conducted according to the Declaration of Helsinki (1964). All participants gave their informed consent prior to their inclusion in the study. **Prior to data collection this investigation was registered on the clinicaltrials.gov website with the following identification number: NCT00981292.**

Treatments

During the three study visits participants received three single-dose treatments in an order dictated by random allocation to a counterbalancing (Latin Square) order.

The three treatments comprised two capsules each containing either 135 mg EGCG (94% pure EGCG plus 6% excipients - DSM Nutritional Products) or an inert placebo. The capsules were combined to give the following treatments:

i) Inert placebo

ii) 135mg EGCG

iii) 270mg EGCG

As this study was the first to investigate the cognitive and CBF effects of EGCG in humans, the doses utilized here are exploratory but represent the quantity of EGCG one might expect to achieve from ~1.5- 3 cups of green tea respectively. This is based on the approximation that 1 cup of green tea will yield ~90mg EGCG (Wu and Wei, 2002).

The treatments were administered in identical size 0 gelatine capsules, which were prepared and coded by a third party who had no further involvement in any aspect of the study. No member of the investigational team was aware of the contents of the capsules until a blind-data review was completed.

Near Infrared spectroscopy (NIRS):

NIRS is a non-invasive brain imaging technique in which two nominal wavelengths of light (~765 and 855 nm), which are differentially absorbed by oxygenated and deoxygenated haemoglobin respectively, are introduced through the skull via a laser emitter and measured, following transit through the upper surface of the cortex, by an optode placed at a pre-set distance from the light source (4cm in this case). Relative changes in the absorption of near infrared light were measured at a time resolution of 10 Hz using a 12 channel Oxymon system (Artinis Medical Systems B.V.). The differential path-length factor was adjusted according to the age of the participant. Relative concentration changes in haemoglobins were calculated by means of a modified Beer-Lambert law (Obrig and Villringer, 2003) using the proprietorial software. The concentration changes in the total levels of haemoglobin (total-Hb) were derived by summing the concentration changes in oxygenated (oxy-Hb) and deoxygenated (deoxy-Hb) haemoglobin. NIRS haemodynamic parameters have previously been shown to correspond strongly with the functional magnetic resonance imaging (fMRI) blood oxygen level dependent (BOLD) signal (Huppert et al., 2006; Steinbrink et al., 2005). NIRS has been used extensively as a technique for multiple-channel imaging of task related brain activity over relevant areas of the head (Schecklmann et al., 2008), including in groups suffering from potential decrements in CBF (Schecklmann et al., 2007).

In this study, given the extended recording period and the investigational aims, a simple two emitter/optode pair configuration was utilised (i.e. 2 channels). The emitter/optode pairs were positioned over the left and right frontal cortex using a standard optode holder headband, which separated the pairs from each other by 4cm. Each pair therefore collected

data from an area of prefrontal cortex that included the areas corresponding to the International 10-20 system Fp1 ad Fp2 EEG positions.

The NIRS data output was time stamped at the start of each task segment to assure that data corresponded to the relevant epoch of task performance.

Cognitive tasks:

In order to assess the contribution that activation of the brain region under investigation made towards any treatment related effects on CBF, a selection of tasks that engender either higher or lower activation of the frontal cortex were employed. **This paradigm was adopted, in part, to assess the differential CBF effects elicited by task demand (with frontal cortex CBF observed to increase with cognitive workload (Son et al., 2005; Tsujimoto et al., 2004)) and how this interacted with treatment, but also in order to elicit greater cognitive demand in the young participants employed here, who are presumably at their cognitive peak. Thus any treatment related cognitive effects are liable to be subtle and likely amplified under greater cognitive demand.** The 'low activation' tasks comprised two simple reaction time tasks (Odd-ball reaction time and Simple reaction time). The 'high activation' tasks (Serial subtractions, Rapid Visual Information Processing, and Stroop tasks) all entail a higher cognitive workload and have all been shown to increase activity specifically in the prefrontal cortex (Schroeter et al., 2002; Drummond et al., 1999; Bench et al., 1993; Lawrence et al., 2002).

The computerised battery of cognitive tasks comprised:

Serial subtractions: This task (for further details see: (Kennedy et al., 2008)), **at baseline, comprised 2 minutes of Serial 3s followed by 2 minutes of serial 7s subtractions with the task length extending to 3.5 minutes for each task at post dose**. At the start of each **subtraction task** a standard instruction screen informed the participant to mentally count backwards in 3s or 7s, as quickly and accurately as possible, using the keyboard's linear number keys to enter each response. Participants were also instructed verbally at the outset that if they were to make a mistake they should carry on subtracting from the new incorrect number. A random starting number between 800 and 999 was presented on the computer screen, which was cleared by the entry of the first response. Each three-digit response was represented on screen by an asterisk. Pressing the enter key signalled the end of each response and cleared the three asterisks from the screen. Performance data (total number of subtractions and number of errors) were calculated for the Serial 3s and 7s elements separately. In the case of incorrect responses, subsequent responses were scored as positive if they were correct in relation to the new number.

Oddball reaction time task: Two distinct visual stimuli (150 x 150 pixels) were presented on screen at regular intervals. The frequent non-target stimuli (n = 200) were red circles and the less frequent target stimuli (n = 40) were green squares. There was also an infrequent distracter in the form of a blue triangle. The frequent and rare stimuli were randomly presented during the task with each presentation consisting of a blank screen for 100 ms, stimulus presentation for 100 ms and finally a blank screen for 1600 ms. During the task the participants had to ignore frequent non-target stimuli and respond by pressing the 'space bar' on a standard computer keyboard when the less-frequent target stimuli **(i.e. the green**

square) appeared. This task is scored for reaction time to target stimuli **and lasted ~3 minutes and 30 seconds at baseline and 7 minutes and 30 seconds at post dose**.

Rapid Visual Information Processing task [RVIP]: This 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 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 space bar as quickly as possible. The task is continuous and lasted for 2 minutes at baseline and 7 minutes at post dose, with 8 correct target strings being presented in each minute. The task is scored for number of target strings correctly detected, average reaction time for correct detections, and number of false alarms.

Stroop task: Participants were required to complete a computerised version of the classic Stroop task in which words describing colours (red, blue, yellow, green) were presented either in the same 'congruent' font, or a different 'incongruent' font. Participants had to identify the colour of the font that the word was written in, rather than the colour that the word was describing, via a response box with coloured keys. For example, if the word red appeared on the screen and was written in a blue font then the correct response would be to press the blue key. **This task lasted for 2 minutes at baseline and 7 minutes at post dose**. The outcomes for the task were the number of correct responses and the average response times for congruent and incongruent stimuli.

Simple reaction time: The participant was instructed to continuously monitor the laptop screen and to press the space bar as quickly as possible every time a single stimulus (upwards pointing arrow) was presented. **The task length was 2 minutes at baseline and 7**

minutes and post dose and the task outcome was average reaction time (msec) response to the stimuli.

Mood Visual Analogue Scales (Mood VAS): Prior to completing baseline and post dose tasks participants were required to rate how ' relaxed', 'alert', 'jittery', 'tired', 'tense' and 'mentally fatigued' they felt by placing a cross with the mouse and cursor on a ~100mm on-screen line between the descriptors 'not at all' and 'extremely'. They also rated their 'overall mood' on a scale anchored by 'very poor' to 'very good' and their levels of 'headache' between 'not at all' and 'extremely'. The VAS were scored as % along the line denoting more of the relevant adjective.

Procedure:

Each participant was required to attend the laboratory on four occasions. The first of these was an initial screening/training visit during which participants provided written informed consent, were screened with regards the study exclusion/inclusion criteria, briefed with regards compliance requirements, and given training in completing the cognitive tasks. This visit was followed within 14 days by the first of three active study mornings.

On each of the three active study mornings, which were conducted 7 days apart, participants attended the laboratory at 11:00am or 2:45pm having eaten nothing in the preceding 2 hours and provided confirmation of continued compliance with the inclusion/exclusion requirements. After a 5 minute seated resting period heart rate was measured and a blood pressure reading was taken, after which participants completed Mood VAS. Following a three minute resting period participants were verbally instructed to

start the period of baseline cognitive task performance which comprised shortened versions of the cognitive tasks: Serial subtractions (3s - 2 mins, 7s – 2 mins); Oddball reaction time task (3 minutes and 30 seconds); Stroop task (2 mins); Simple reaction time (2 mins) and RVIP (2 mins). Participants then rested for 5 minutes after which they consumed their treatment for that day, following which they sat quietly, watching one of a selection of non-arousing DVDs, during a 45 minutes 'absorption' period. **The T_{max} of green tea polyphenols varies hugely in the literature, ranging from 0.5- (Leenen et al., 2000; Pietta et al., 1998) to 4 hours (Nakagawa et al., 1997) depending on the form of catechin/s. Huge individual variability in metabolism also influences T- and C_{max}. Thus, in the absence of consistent bioavailability information, 45 minutes was chosen as a somewhat exploratory absorption period.**

After completing the Mood VAS again participants were verbally instructed to start the period of post dose task performance which comprised six 7 minute epochs of individual task performance (plus up to an additional 30 seconds for those tasks that depended on speed of response). The task order was Serial subtractions (3s then 7s - 3.5 minutes each), Oddball reaction time task (7 minutes and 30 seconds), RVIP, Oddball reaction time task, Stroop task, and then the Simple reaction time task (all 7 minutes) (i.e. ~ 42 minutes of continuous task performance). After a 3 minute rest period heart rate was measured and a blood pressure reading was taken from participants. NIRS data was captured throughout. The timelines and running order of the testing session are shown in Figure 1.

Figure 1 about here

Statistics

The analyses of NIRS data were conducted with Minitab 15 for Windows (Minitab Inc, State College, PA) and behavioural data with SPSS 16.0 for Windows (SPSS Inc, Chicago, IL). NIRS data was converted to 'change from baseline' (calculated from the final 3 minutes of the 5 minute resting period immediately pre-treatment) and data collected from the left and right hemispheres were averaged. Data from the 'resting/absorption' period (minutes 1 to 45) and the task performance period (minutes 46 to 87) were analyzed separately. Data from the 'resting/absorption' period was averaged across 6 equal 7.5 minute epochs and analysed by two-way repeated measures ANOVA (epoch x treatment). Data from the task period data was averaged across the 7 min task period, also giving 6 epochs, each of 7 minutes duration. This data was analysed by two-way repeated measures ANOVA (task [epoch] x treatment). In the case of those analyses that showed a significant main effect of treatment or a task/epoch x treatment interaction, planned comparisons of data from each task or epoch were then made between placebo and each of the EGCG treatment groups using t tests calculated with the Mean Squares Error from the ANOVA. A Bonferroni adjustment was made for multiplicity (Kepple, 1991). As the duration of each complete epoch of averaged NIRS data entered into the analysis was substantially longer than the potential physiological oscillations that can cause drift in shorter periods of NIRS recording (Hoshi, 2007) no adjustment was required to control for this phenomena.

Prior to the primary analyses, the distribution of the NIRS data was found to have a significant negative skew. The data was therefore normalised by inversion followed by a

Log(10) transformation. A further ANOVA was then conducted for each measure to confirm that any significant differences found on the ANOVAs of raw data were retained following normalisation.

Task performance data were analysed by within subjects ANCOVA (treatment) with pre-treatment performance included as a co-variate for each individual task/measure (Serial 3s, Serial 7s, RVIP, Stroop, Simple reaction time, Oddball and Mood VAS).

Blood pressure data (systolic, diastolic and heart rate) were analysed by within subjects ANOVA (treatment x pre-post treatment).

RESULTS

Cognitive performance and mood

No significant treatment related differences were observed on any of the cognitive or mood measures.

Heart rate and blood pressure

There was a significant reduction in heart rate across conditions between the initial pre-dose measurement and the measurement taken at the end of testing (approximately 90 minutes post-dose) [$F(1, 26) = 8.15$, $p < 0.05$]. There was no interaction between treatment and assessment ($F < 1$).

NIRS

Deoxygenated haemoglobin: There were no significant differences in terms of deoxy-Hb during either the resting or task performance periods.

Oxygenated haemoglobin: There was no effect of treatment during the absorption period. However, on the initial ANOVA, there was a significant main effect of treatment on oxy-Hb levels during the task period [$F(2, 52) = 3.41$, $p < 0.05$]. The analysis of normalised data confirmed that this effect survived log transformation [$F(2, 52) = 3.19$, $p < 0.05$]. Reference to the planned comparisons of the raw data means from each epoch showed that, whereas there were no significant differences associated with the higher dose of EGCG, the lower dose lead to a reduction in oxy-HB during each of the six 7 minute task periods [t's (260) = 4.83 – 5.84, all $p < 0.01$].

Total levels of haemoglobin: Similarly, there was no effect of treatment on total-Hb during the absorption period. However, on the initial ANOVA, there was a significant main effect of treatment on total-Hb during the task period [$F(2, 52) = 3.53$, $p < 0.05$]. The analysis of normalised data confirmed that this effect survived log transformation [$F(2, 52) = 3.38$, $p < 0.05$]. Reference to the planned comparisons of the raw data means from each epoch showed that, whereas there were no significant differences associated with the higher dose of EGCG, the lower dose lead to a reduction in oxy-HB during each of the six 7 minute task periods [t's (260) = 4.96 – 6.85, all $p < 0.01$].

There was no interaction between task and treatment with regards any of the NIRS parameters. A graphic representation of the mean total-Hb response to the first three

minutes of task performance (averaged across tasks) are shown in Figure 2. The mean concentration changes (plus SEM) during the absorption and task periods for deoxy-Hb, and total-Hb are shown in Figure 3.

Figure 2 and Figure 3 about here

DISCUSSION

The results here show that, in comparison to placebo, the consumption of EGCG resulted in modulation of CBF parameters in the frontal cortex during task performance. This effect was restricted to the lower (135 mg) dose of EGCG and was seen as a reduction in CBF during the task period, as indexed by the concentration of haemoglobin (total-Hb), with an identical pattern of results seen in terms of oxy-Hb. With regards deoxy-Hb, no effect of treatment was observed. These CBF effects were not associated with any significant modulation of either cognitive performance or mood and, whilst they were only evident during the task performance period, there was no indication that the treatment related effects were predicated on the amount of activation of the frontal cortex associated with the tasks that were assumed to engender 'high' and 'low' activation of this brain region.

Whilst the results here show clearly that EGCG can modulate aspects of brain function, in the absence of any modulation of behavioural parameters it is difficult to say whether the comparative reduction in CBF seen here represents a net benefit or decrement in terms of brain function. On the most simplistic level optimal performance should require increased

delivery of blood, and therefore metabolic substrates, during neuronal demand. **A recent electroencephalography (EEG) study investigating the electrical brain response to EGCG in healthy human participants, however, corresponds with the results presented in this paper; finding that although 300mg orally consumed EGCG evinced increased cerebral activity in the form of alpha, beta and theta waves, no task performance effects were observed (Scholey et al., In press).**

Overall CBF has been shown to decline throughout adulthood (Parkes et al., 2004), and both reduced basal CBF (Bertsch et al., 2009) and task specific CBF responses (Sorond et al., 2008) are associated with declining performance during healthy ageing. Similarly, compromised CBF has also been suggested as a key causal factor in the reduced cognitive function seen with age and in a number of neurodegenerative diseases (Farkas et al., 2002). However, whilst the reduced CBF seen here may therefore be interpreted as a detrimental effect it may also reflect a reduced requirement for blood flow due to a further unidentified factor improving other aspects of brain function. An alternative possibility is that the reduced CBF might be negative in itself, but is compensated for by another, unmeasured mechanism. As an example of this, caffeine is a vasoconstrictor that has been shown to reduce CBF as assessed by a number of methods (Field et al., 2003; Lunt et al., 2004; Sigmon et al., 2009) including the NIRS methodology employed here (Kennedy and Haskell, 2011). In the case of caffeine the vasoconstriction is predicated on the blockade of adenosine A_{2A} receptors throughout the cerebro-vascular system, whilst concomitant blockade of A_1 receptors throughout the brain increases neuronal firing rate (Laurienti et al., 2003), leading ultimately to net improvements in alertness and attention task performance despite reduced CBF.

In terms of specific mechanisms underlying the effects seen here, it is interesting to note that in a similar study we previously assessed the effects of single doses (250 mg, 500 mg) of the polyphenol resveratrol in healthy human participants and observed a linear, dose-related increase in CBF in the frontal cortex, during the performance of the mentally demanding, 'frontal' Serial subtraction and RVIP tasks (Kennedy et al., 2010). The opposite effect on CBF seen here following EGCG may well be related to differential effects on the vaso-relaxatory process associated with NOS. In the case of resveratrol, evidence suggests that it increases NO synthesis via eNOS (Tsai et al., 2007) and nNOS (Hung et al., 2004) and consequently has evinced consistent effects in terms of NO related peripheral vaso-dilation in animals (Lekakis et al., 2005; Zou et al., 2003) and humans (Wong et al., 2011) and increased CBF in rats (Lu et al., 2006) and humans (Kennedy et al., 2010). EGCG on the other hand has a less clear cut pattern of effects on vascular/microvascular tone, with some evidence of improved peripheral endothelial function in humans (Widlansky et al., 2007) but demonstrations *ex vivo* of both vaso-constriction (Sanae et al., 2002) and dilation (Alvarez et al., 2006). It has also been shown to up-regulate eNOS (Widlansky et al., 2007) but inhibit nNOS (Lin and Lin, 1997). Whilst both eNOS and nNOS are both found in brain tissue (Toda and Okamura, 2003), it is nNOS derived NO which has been observed to make the greatest contribution to activity dependent vasodilation (Santizo et al., 2000). Interestingly, the nNOS isoform has also recently been associated with peripheral vasodilatory responses (Melikian et al., 2009) including that elicited by cognitive task performance (Stroop) in healthy human participants (Seddon et al., 2008). EGCGs inhibition of this isoform might therefore underlie the less than straightforward effects seen, both in terms of the literature pertaining to peripheral vasodilation, and the potential cerebral vasoconstriction see in the current study.

This same inconsistency may also explain the dose specific effect seen here. EGCG's effects on vascular tone have been shown to differ both over time and with dose. So, for instance, EGCG has been shown to have transient peripheral vasoconstricting effects, followed by vasodilation (Alvarez et al., 2004), and animal data confirms that at low doses green tea catechins have vasoconstricting properties (Shen et al., 2003) and that at higher doses they induce vasorelaxation (Lorenz et al., 2004). Whilst the differential effects of EGCG on the eNOS and nNOS isoforms makes it difficult to extrapolate CNS effects from the peripheral data it may well be that the lowest dose has cerebral vasoconstriction properties that are simply not seen at the higher dose, and that a further increase in dose may result in increased CBF. Naturally these possibilities will need to be explored further, and it would be prudent to investigate longer time courses and both lower and higher doses in order to document the full profile of EGCG's vasoregulatory properties. Interestingly, the migraine treatment Sumatriptan also functions via modulation of NO, demonstrating both vasorelaxing and a vasoconstricting properties (Elhusseiny and Hamel, 2001), and does not have a straightforward dose response profile (Fowler et al., 1991). It is also interesting to note that evidence from dose ranging studies in humans shows that a wide range of phytochemicals and herbal extracts have greater impacts on cognitive performance and physiological parameters at lower, rather than higher, doses. Recent examples of such findings from dose ranging studies include sage (Scholey et al., 2008; Tildesley et al., 2003), **guaraná** (Haskell et al., 2007), cocoa- flavanols (Scholey et al., 2010) and ginseng (Reay et al., 2005).

One aspect of the current study that was somewhat disappointing was the failure to demonstrate clear, differential haemodynamic responses to the more demanding 'high

activation' and less demanding 'low activation' tasks, whereby the former might have been expected to engender higher total-Hb and lower deoxy-Hb. This manipulation was included to try to differentiate whether any treatment related effects on CBF parameters were specifically related to the level of activation, and therefore NO driven increases in blood flow requirements, of the brain region. However, there was no evidence of any treatment/task interaction, and the main effect of EGCG on CBF would appear, from the data here, to be a general one across tasks, irrespective of cognitive workload. Reference to Figure 3 shows a simple pattern of declining concentrations of total-Hb throughout the task period, irrespective of treatment. **This apparent lack of sensitivity to task intensity could be as a consequence of only utilizing a 2 channel NIRS system, thus reducing spatial resolution. Future studies might therefore consider using additional channels and recording at other locations of the cortex to assess if supplementary regions are involved in the completion of these tasks.**

Task length and/or perception of demand/difficulty could also explain the lack of CBF sensitivity to tasks. Reference to the finer grained analysis shown in Figure 2, which presents the total-Hb data (averaged across tasks) for the first three minutes of task performance in 10 second epochs, shows that total-Hb underwent the expected increase during the first minute of performing each task, but that this was followed by a gradual decline throughout the remainder of each task. This does suggest that the overall pattern of haemodynamic responses was due to the extended length of the tasks employed, and that employing shorter, more demanding tasks may be a more effective approach to engendering a long period of increased haemodynamic response. Alternatively, it is also notable that heart rate fell significantly across all three conditions from pre-dose to 90

minutes post-dose and it is possible that this reflects a simple reduction in physiological arousal between arriving at the laboratory and completing the assessment. Future research using this method of assessing CBF might usefully include an extended rest period before recording commences, with heart rate monitoring throughout.

One key issue regarding EGCG and other polyphenols is that of the low bioavailability of the parent compound (Lee et al., 2002), which questions the relevance to humans of much of the *in vitro* research conducted with concentrations of EGCG that would not be attainable in mammals. The paradoxical finding of multifarious *in vivo* effects despite this poor bioavailability suggests that the metabolites of EGCG (and other polyphenols) may be active in their own right: the substantial amounts of two catechin metabolites (4'- *O*- MeEGC, (-) - 5- (3', 4', 5'- trihydroxyphenyl) - γ- valerolactone (M4) and (-) -5- (3', 4'- dihydroxyphenyl) - γ- valerolactone (M6)) detected in human plasma and urine would certainly suggest that they could possess biological activities (Lee et al., 2002). Alternatively EGCG may operate principally via secondary mechanisms, for instance NO synthesis, and as such only relatively small concentrations may be required to instigate a cascade of cellular events. In this respect it is interesting to note that EGCG has been shown to cross the blood-brain barrier in rodents (Suganuma et al., 1998). Further research is certainly required to fully explore the biotransformation of polyphenols in humans in order to better appreciate their sources of bioactivity.

In conclusion, the results here demonstrate that the green tea catechin EGCG is able to modulate CBF in healthy human participants, with decreased concentrations of haemoglobin seen in the frontal cortex during cognitive task performance following the lower (135 mg) of two doses that were administered. However, the lack of effects on

cognitive performance and mood make it difficult to interpret the modulation in CBF as either a positive or negative modulation of overall brain function. With these results in mind, future research might usefully investigate the effects of both lower and higher doses of EGCG on both cerebral and peripheral blood flow. The combination of methodologies may elucidate the comparative roles of eNOS and nNOS in any vaso-regulatory properties of EGCG. **It would also be prudent to image other regions of the brain during the performance of 'frontal' tasks and ascertain whether other areas of the brain are involved and sharing the burden of increased workload.** Investigating the effects of longer treatment periods and the behavioural/CBF effects of this polyphenol in cohorts that may be more susceptible to modulation would also seem to be warranted by the current investigation.

Author Statement: None of the authors has any conflict of interests with regards the above paper. This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors. All materials were purchased on the open market. All of the authors contributed to and reviewed the manuscript. DK and CH designed and supervised the study. DK conducted the statistical analysis. JF and RV collected the data. EW was the principal author.

Abbreviations: ANOVA - Analysis of Variance; BOLD - blood oxygen level dependent; CBF - cerebral blood flow; deoxy-Hb - deoxygenated haemoglobin; EGCG - Epigallocatechin gallate; eNOS - endothelial nitric oxide synthase; fMRI - functional magnetic resonance imaging; iNOS - inducible nitric oxide synthase; NIRS - Near Infrared Spectroscopy; NO - nitric oxide; nNOS - neuronal nitric oxide synthase; oxy-Hb - oxygenated haemoglobin; total-Hb - total levels of haemoglobin; VAS - Visual Analogue Scales.

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Figures:

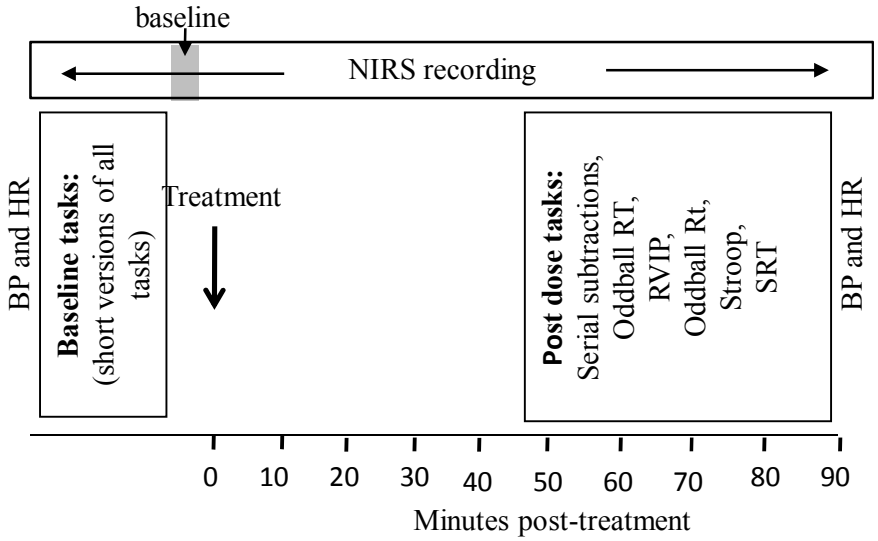

Figure 1. Timelines of each assessment. Blood pressure and heart rate were measured. Participants then completed one repetition of the tasks to establish baseline performances. After a 5 minute resting period, they received their day's treatment and, after a 45 minute resting/absorption period, they completed a longer version of each of the baseline tasks. After completion heart rate and blood pressure were measured again. Near- infrared spectroscopy (NIRS) data were collected throughout with the pre-treatment resting phase used to baseline- adjust all pre-treatment data.

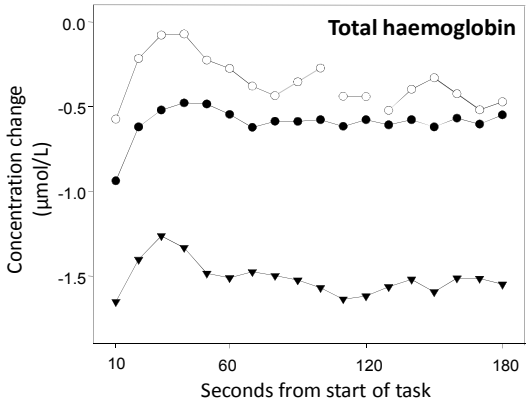

Figure 2. Total-Hb data, averaged across the tasks, and shown for the first three minutes of task performance in 10 second epochs.

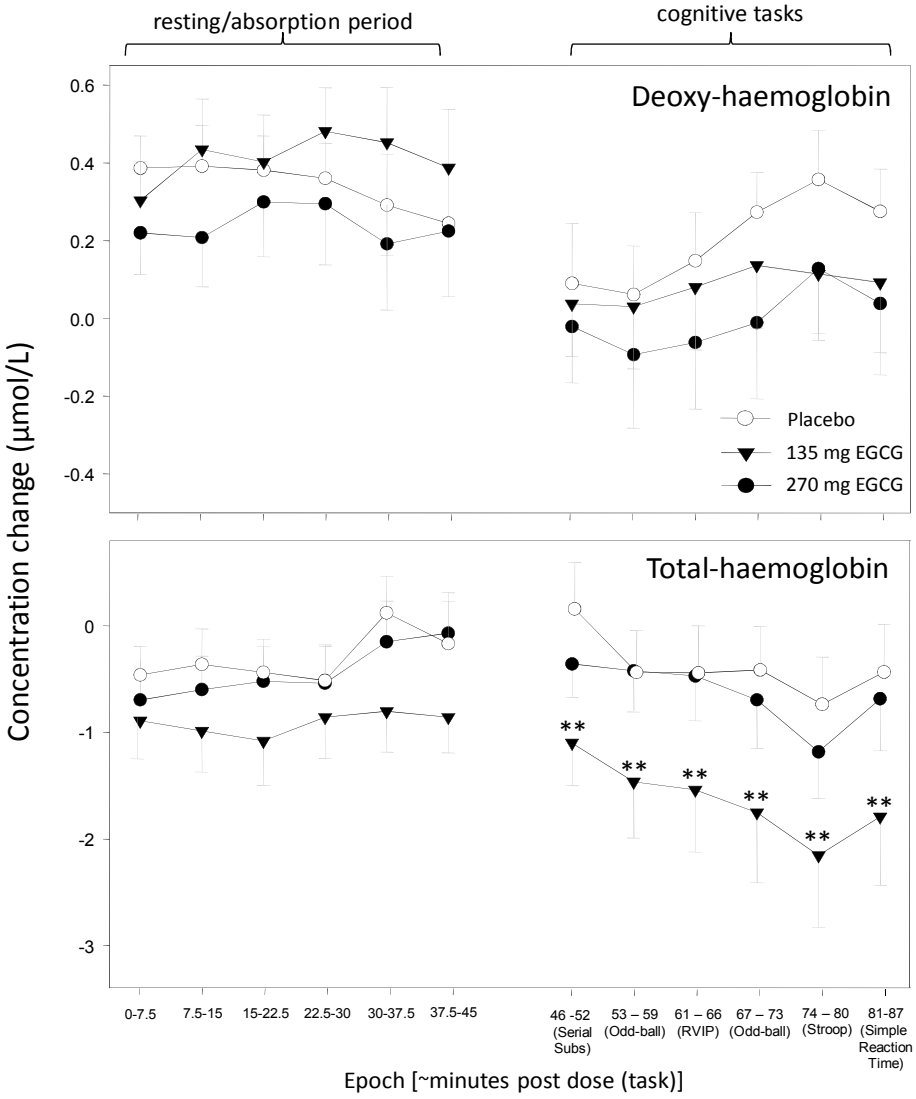

Figure 3. Mean (plus SEM) changes in concentrations of deoxy-Hb and Total-Hb during the absorption period and six 7 minute task periods (** = $p < 0.01$, Bonferroni adjusted planned comparisons).