Cerebral blood flow and behavioural effects of caffeine in habitual and non-habitual consumers of caffeine: A Near Infrared Spectroscopy study

Running title: Haemodynamic effects of caffeine

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ABSTRACT

Caffeine has been shown to modulate cerebral blood flow, with little evidence of tolerance to these effects following habitual use. However, previous studies have focused on caffeine levels much higher than those found in dietary servings and have compared high caffeine consumers with low consumers rather than 'non-consumers'. The current placebo-controlled double-blind, balanced-crossover study employed Near Infrared Spectroscopy to monitor pre-frontal cerebral-haemodynamics at rest and during completion of tasks that activate the pre-frontal cortex. Twenty healthy young habitual and non-habitual consumers of caffeine received 75mg caffeine or placebo. Caffeine significantly decreased cerebral blood flow but this was subject to a significant interaction with consumption status, with no significant effect being shown in habitual consumers and an exaggerated effect in non-habitual consumers. These findings suggest that caffeine, at levels typically found in a single dietary serving, is able to modulate cerebral blood flow but these effects are subject to tolerance.

Key words: caffeine, cognitive, cerebral blood-flow, near infrared spectroscopy, NIRS, haemodynamics, consumers, habituation

INTRODUCTION

Blockade of adenosine receptors by caffeine leads to increased alertness (Quinlan et al., 2000; Smit & Rogers, 2000; Smith, Sturgess, & Gallagher, 1999) and improvements to cognition, particularly reaction time (Durlach, Edmunds, Howard, & Tipper, 2002; Smit & Rogers, 2000) and vigilance (Brice & Smith, 2001; Childs & de Wit, 2006). It has been suggested that these effects merely represent alleviation of withdrawal in habitual caffeine consumers (James, 1994). However, comparisons of low/non caffeine consumers' and regular consumers' behavioural responses to caffeine have demonstrated similar effects irrespective of habitual caffeine intake (Richardson, Rogers, Elliman, & Odell, 1995; Rogers, Martin, Smith, Heatherley, & Smit, 2003) even when there is no evidence of withdrawal in the habitual consumers (Addicott & Laurienti, 2009; Haskell, Kennedy, Wesnes, & Scholey, 2005; Hewlett & Smith, 2006) and positive effects of dietary levels of caffeine have been shown in non/low consumers (Adan & Serra-Grabulosa, 2010). Studies examining the effects of caffeine in non-withdrawn consumers have typically produced mixed results (Heatherley, Hayward, Seers, & Rogers, 2005; Hewlett & Smith, 2006; Warburton, 1995; Yeomans, Ripley, Davies, Rusted, & Rogers, 2002)..

In terms of vascular effects, caffeine exerts a complex pattern of effects that include endothelium-dependent vasodilation via modulation of nitric oxide synthesis and vasoconstriction mediated by adenosine receptor antagonism (Umemura et al., 2006). However, the net effect is reduced cerebral blood flow (CBF). This has been seen in the modulation of putative indices of CBF using a number of methodologies. These include Positron Emission Tomography (PET) (Cameron, Modell, & Hariharan, 1990); Xenon Clearance (Lunt, Ragab, Birch, Schley, & Jenkinson, 2004), Trans-cranial Doppler (Jones, Herning, Cadet, & Griffiths, 2000; Sigmon, Herning, Better, Cadet, & Griffiths, 2009) and Magnetic Resonance Imaging (MRI) (Field, Laurienti, Yen, Burdette, & Moody, 2003). With regards the latter method, Field et

al. (2003), using quantitative perfusion MRI undertaken approximately 60 to 90 minutes post-dose of placebo or caffeine, found that habitual high consumers (>300mg/day) had greater placebo CBF in comparison to lower consumers (<125mg/day) following abstention for 30 hours. Cerebral blood flow was lower across groups following 250mg caffeine than following placebo, with a significantly greater reduction in perfusion seen following caffeine in low consumers. Similarly, Addicott et al. (2009) assessed the effects of 250mg caffeine and placebo on grey matter perfusion 1.5 to 2 hours post-dose in lower (<200mg), moderate (>200<600mg) and higher consumers (>600mg) following either 30 hours of caffeine withdrawal or maintenance. They found greater perfusion following abstention in high as opposed to low consumers, but no significant difference in the extent to which caffeine reduced CBF across consumption groups following withdrawal. However, CBF was significantly reduced following caffeine for the low consumers (in comparison to high) when assessed during maintenance of normal daily caffeine habits. Taken together, these studies both provide evidence of higher cerebral perfusion in higher habitual consumers of caffeine in a state of withdrawal. Field et al. (2003) also demonstrated a lowered CBF response to caffeine in higher habitual consumers, whereas this effect was only apparent when consumers were non-withdrawn in Addicott et al. (2009). A number of calibrated (to hypercapnia) BOLD fMRI studies have also shown that a high single dose of caffeine (~200mg+), administered to habitual consumers uncouples the relationship between CBF and local oxygen consumption by reducing blood flow, but not oxygen metabolism (Chen & Parrish, 2009a; Perthen, Lansing, Liau, Liu, & Buxton, 2008). Caffeine has also been shown to modulate neuronal activity in brain areas associated with executive and attentional processes during performance of a verbal working memory task by moderate caffeine consumers (Koppelstaetter et al., 2008).

Whilst caffeine withdrawal and administration in habitual consumers have been shown to have reliable opposite effects on parameters related to cerebral blood flow, little research has

addressed the question of differential effects in habitual consumers and non-habitual consumers of caffeine. In addition, studies in this area have focused on the effects of 200mg+ of caffeine and no study to date has assessed the effects of a dose of caffeine equivalent to a single dose that would be encountered in everyday life. As an example, a single serving of coffee will typically contain approximately 75mg of caffeine, and the average intake of caffeine for a full day is in the region of 200mg in the UK (Brice & Smith, 2002).. Given that findings from studies of higher doses may bear little relevance to naturalistic doses it is important to study the effects of levels of caffeine commonly found in the diet. Similarly, the time course of the initial cerebral haemodynamic effect of caffeine remains unexplored, with previous studies simply taking a 'snap shot' at a single time-point post-dose.

The aim of the present study was therefore to further explore the cerebral haemodynamic effects of caffeine across habitual consumers and non-habitual consumers of caffeine employing a double-blind, placebo-controlled methodology. The 75mg caffeine dose examined represents the caffeine level found in a typical dietary serving of coffee (Gray, 1998). In order to examine cerebral haemodynamics from pre-dose and throughout the 66 minute testing session Near Infrared Spectroscopy (NIRS) of the pre-frontal cortex was employed. NIRS is a well-validated non-invasive brain imaging method, predicated on the intrinsic optical absorption properties of oxygenated and deoxygenated haemoglobin, which measures cerebral blood flow (total levels of haemoglobin) in the surface layers of the cortex. In addition, cognitive performance was assessed using tasks that activate the pre-frontal cortex and subjective mood, blood pressure and heart rate were assessed both at baseline (to assess withdrawal effects) and following caffeine ingestion.

MATERIALS AND METHODS

Design

A placebo-controlled, double-blind, balanced crossover design was employed. The study received ethical approval from Northumbria University's School of Psychology and Sport Sciences Ethics Committee and was conducted according to the Declaration of Helsinki (1964).

Initial screening

Participants were initially recruited on the basis of their responses to three questions assessing average daily consumption of tea, coffee and soft drinks. Potential volunteers were then asked to complete a questionnaire assessing daily caffeine consumption. 'Non-habitual consumers' were defined as those who refrained from drinking tea or coffee and who consumed ≤ one caffeinated soft drink per day (mean 27mg caffeine/day; range 6–67mg). 'Habitual consumers' were defined as those who consumed ≥3 cups of tea and/or coffee per day (mean consumption 333mg/day, range 150–515mg).

Exclusion criteria included: lack of proficiency in English to the level of a native speaker; pregnancy or seeking to become pregnant; currently taking food supplements, illicit drugs or medication (including the contraceptive pill); food allergies or intolerances; a Body Mass Index >40; tobacco consumption (even occasionally); a history of, or current, drug or alcohol abuse; head injury, neurological disorder or neuro-developmental disorder.

Participants

Twenty healthy adults (10 males, mean age 21.4 years, range 19-28, 4 left-handed, mean BMI 24.6) took part in the study. Ten participants were 'non-habitual consumers' (5 male,

mean age 21.4 years, range 19-28, 2 left-handed, mean BMI 24.4) and 10 were 'habitual consumers' (5 male, mean age 21.3 years, range 19-26, 2 left-handed, mean BMI 24.9).

Treatments

Each participant in each group received each of the treatments on separate days, not less than 48 hours apart, in an order dictated by random allocation to a counterbalancing schedule. Depending on the condition to which the participant was allocated on each day they received either: Inert placebo; or 75 mg caffeine hydrochloride BP (Fisher Scientific UK, Leicestershire). In order to maintain the double-blind procedure the treatments were prepared and coded by a third party who had no further involvement in any aspect of the study and each treatment was administered in size 1 gelatine capsules. Chi-square analysis of data relating to the participant's belief about the treatment administered during their second visit revealed that participants were not aware of which treatment they had received.

Physiologic measures

Heart and blood pressure were monitored using the Boso medicus prestige (Bosch and Sohn, Jungingen, Germany). This is a fully automatic upper arm monitor that measures heart rate (bpm) and systolic and diastolic blood pressure (mmHg).

Cognitive and mood measures

All cognitive and mood measures were delivered using the Computerised Mental Performance Assessment System (COMPASS), a purpose designed software application for the flexible delivery of randomly generated parallel versions of standard and novel cognitive assessment tasks that has previously been shown to be sensitive to nutritional interventions (Kennedy, Veasey et al., 2010; Kennedy, Wightman et al., 2010).

Seven visual analogue scales that have previously been used in caffeine research (Rogers et al., 2003) with the addition of a single 'mental fatigue' visual analogue scale were utilised (Haskell, Kennedy, Milne, Wesnes, & Scholey, 2008; Haskell et al., 2005). An adjective appeared on screen and the participant positioned a cross on a line indicating how they currently felt between 'not at all' (left-hand) and 'extremely' (right-hand) for the adjectives 'relaxed', 'alert', 'jittery', 'tired', 'tense', 'headache' and 'mental fatigue' or between 'very good' (left-hand) and 'very bad' (right-hand) for 'overall mood'. These scales were scored individually as % percent along the line from left to right.

The three cognitive tasks that form the Cognitive Demand Battery (CDB) have previously been shown to activate the pre-frontal cortex in brain imaging studies (Drummond et al., 1999; Lawrence, Ross, & Stein, 2002; Kazui et al., 2000). The objective of this collection of tasks is to assess the impact of the treatment on speed/accuracy and mental fatigue during continuous performance of cognitively demanding or 'effortful' tasks. Multiple completions of the nine minute battery of tasks (comprised of 4 minutes of serial subtractions [2 mins of serial 3s subtractions and 2 mins of serial 7s subtractions]; and 5 minutes of Rapid Visual Information Processing has previously been shown to reliably increase self-ratings of 'mental fatigue' and to be sensitive to a number of natural interventions (Kennedy et al., 2008; Kennedy & Scholey, 2004; Reay, Kennedy, & Scholey, 2005, 2006). The components of the CDB are described below.

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 linear 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 the total number of 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. The duration of this task was 2 min.

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.

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.

'Mental fatigue' and 'Difficulty' Visual Analogue Scales (VAS): Following each repetition of the cognitive tasks participants rated their current subjective mental fatigue state and how difficult they found the tasks by making a mark on computerised VAS with the end points labelled 'not at all' (left hand end) and 'extremely' (right hand end).

Cerebral blood flow – Near Infrared Spectroscopy (NIRS)

NIRS is a non-invasive brain imaging technique in which two nominal wavelengths of light (in this case using the manufacturer's settings of ~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 pathlength factor (an adjustment that takes into account age-related changes in the optical path-length through tissue) was set according to the age of the participant. Relative concentration changes in haemoglobins were then calculated by means of a modified Beer–Lambert law

(Obrig & 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 and deoxygenated haemoglobin thereby giving a measure of the overall levels of haemoglobin (and therefore blood volume/flow) in the investigated cortical tissue.

Individual NIRS haemodynamic parameters have previously been shown to correspond strongly with measurements made using fMRI (Huppert, Hoge, Diamond, Franceschini, & Boas, 2006; Steinbrink et al., 2006). However, as well as providing a better proxy measure of pre-frontal cortical blood flow, NIRS has the additional advantages in the current context that it can be used to record continuously for long periods of time and simply requires the participant to be seated, allowing recording during complex computerised tasks that require hand and arm movements. 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 that separated the pairs from each other by 4 cm. Each pair collected data from an area of pre-frontal cortex that included the areas corresponding to the international 10-20 system Fp1 and Fp2 EEG positions.

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

Procedure

Each participant was required to attend the laboratory on three occasions. The first of these was an initial screening/training visit, and this was followed within 14 days by the first active study visit. During the initial visit participants provided written informed consent and were screened with regards the study exclusion/inclusion criteria. Training was given on the cognitive tasks and rating scales and familiarisation with the study procedures was provided. Participants

were also informed that in order to be compliant with the study restrictions they must consume the same breakfast, lunch and snacks at the same times across both study visits; and must not consume any caffeine or alcohol from 9pm the previous day (a diary card was provided to record food and drink intake as well as a list of caffeine-containing food, drink and medication).

On the two active study visits, which were identical with the exception of the treatment administered ,participants were assigned to attend the laboratory at either 12.45pm or 3.00pm on both days and provided confirmation of continued compliance with the inclusion/exclusion requirements and food/caffeine intake restrictions. They also provided a saliva sample using salivettes (Sarstedt, Leicester, UK) for in-house batch analysis using the Emit system (Syva, Palo Alto, USA) to confirm abstinence from caffeine. Prior to taking their treatment for that day participants were fitted with the NIRS headband; had their blood pressure and heart rate recorded; completed mood scales; and completed a single repetition of the CDB in order to establish baseline measurements for physiologic, mood and cognitive parameters. Following this, participants sat quietly for 5 minutes, with the last 3 minutes of this period utilised as the NIRS resting baseline measurement. Participants then consumed their treatment for that day and sat quietly, watching one of a selection of neutral DVDs, during a 25 minute 'absorption' period, chosen to allow a peak during the performance of cognitive tasks (caffeine t_{max} ~30-45 minutes - Fredholm, Battig, Holmen, Nehlig, & Zvartau, 1999). Following this, heart rate and blood pressure were again measured and mood visual analogue scales were completed. They then started the period of post-treatment task performance, and made four consecutive repetitions of the CDB (i.e. 36 minutes of continuous task performance). NIRS data was captured throughout. The timelines and running order of the testing session are shown in Figure 1. At the end of the second study visit participants were asked to indicate whether they thought they had received caffeine or placebo on their second visit before being debriefed and compensated £50 for their time.

Figure 1 about here.

Statistics

The primary analyses of cognitive performance, mental fatigue and 'difficulty' measures were conducted by MANOVA of 'change from baseline data' from individual tasks with treatment and repetition as within-subjects terms and consumer status as a between-subjects term.

The primary analysis of the NIRS total-Hb data was conducted by repeated measures ANOVA with treatment and epoch as within-subjects terms and consumer status as a between-subjects term. Two sets of *a priori* planned comparisons were made to address the specific experimental questions. In order to assess the overall effects of caffeine on CBF (as indexed by total-Hb) comparisons were made between mean data from each epoch for the caffeine and placebo conditions irrespective of habitual/non-habitual consumption group. Additionally, in order to investigate any differential effects between the two consumption groups, comparisons were made separately for the habitual and non-habitual consumption groups on mean data from each epoch for the caffeine and placebo conditions. The planned comparisons were carried out using Bonferroni corrected t tests calculated with Mean Squares Error from the ANOVA and evaluated with the degrees of freedom associated with this error term (Keppel, 1991) with the significance level adjusted for the number of comparisons (epoch x treatment interaction = 13 comparisons; caffeine x treatment x consumption group interaction = 26 comparisons).

Heart rate, blood pressure and mood visual analogue scale outcomes were analysed with mixed-design ANOVA, with assessment (pre/post treatment) and treatment as within-subjects terms and consumer status group as a between-subjects terms.

Only the results of those measures that evinced significant effects are reported below.

RESULTS

Salivary caffeine and demographics

Differences in the demographic and pre-dose salivary caffeine data of the consumer groups (habitual vs. non-habitual consumer) were explored with independent t-tests and chi-square where appropriate. Analysis of pre-dose salivary caffeine levels revealed all participants had complied with caffeine restrictions (habitual mean 0.1 µg/ml; non-habitual mean 0.1 µg/ml). There were no significant consumer status differences in salivary caffeine levels or demographics, with the exception of the expected greater average caffeine consumption in the habitual consumers [$t(19)=6.35$, $p<0.01$].

Pre-treatment differences and consumer status effects in the absence of treatment

Prior to the primary statistical analysis, separate one way, repeated measures ANOVAs of pre-dose baseline mood, cognitive and physiologic data were conducted to ascertain any chance differences across the treatment conditions prior to treatment administration. To assess the possibility that caffeine withdrawal impacts upon these same parameters, one way ANOVAs were conducted to ascertain any consumer status effects (habitual vs. non-habitual consumer) in the absence of treatment. There were no significant differences on any of the measures.

Heart rate, blood pressure and mood

There were no significant effects on any of these measures.

Cognitive Demand Battery

Cognitive demand battery data were analysed as change from pre-dose baseline for each individual task (Serial 3s, Serial 7s, RVIP, ‘mental fatigue’, ‘difficulty’) by repeated

measures MANOVA (treatment group x repetition x consumer status). Baseline and change from baseline data for the CDB are shown in Table 1.

The MANOVA performed for serial sevens (total responses and number of errors) revealed there was a significant main effect of treatment on the performance of the Serial Sevens task ($p<0.05$). A univariate ANOVA revealed this was due to fewer errors being committed following caffeine than placebo [$F(1,17)=9.63$, $p<0.01$, $\eta_p^2=0.36$, observed power=0.83],. There were no other significant differences on the cognitive measures fatigue/difficulty visual analogue scales related to treatment.

Table 1 about here

Near Infrared Spectroscopy

NIRS data was converted to 'change from baseline' (3 minute pre-treatment resting period) and averaged across 5 minute epochs during the 25 minute 'resting/absorption' period, and 4 minute (Serial Subtractions) or 5 minute (RVIP) epochs during the cognitive task performance periods. As the duration of each complete epoch of averaged NIRS data entered into the analysis was substantially longer than the potential physiological oscillations (e.g. heart rate/respiration etc) that can cause drift in short periods of NIRS recording (Hoshi, 2007) no adjustment was required to control for this phenomena. .

Prior to the primary analysis a within subjects Analysis of Variance (ANOVA) was carried out with left/right optode included as an additional factor (hemisphere x treatment group x epoch) to examine any hemispheric differences in response. As there were no treatment related interactions involving this factor the data from the two channels were averaged across hemispheres for the analysis and figures reported below.

There was a significant interaction between treatment group and epoch for cerebral blood flow as indexed by total-Hb [$F(12,216)=1.94$, $p<0.05$, $\eta_p^2=0.97$, observed power=0.91] (see Figure 2). Reference to the Bonferroni adjusted planned comparisons showed that caffeine consumption was associated with significantly reduced concentrations of haemoglobin in comparison to placebo during the last 6 epochs of recording (40-43 min [$t(216)=3.13$, $p<0.05$], 44-48 min[$t(216)=3.08$, $p<0.05$], 49-52 min [$t(216)=3.35$, $p<0.05$], 53-57 min [$t(216)=3.68$, $p<0.01$], 58-61 min [$t(216)=2.19$, $p<0.01$], 62-66 min [$t(216)=2.96$, $p<0.05$]) with a trend towards the same effect during the 31-34 mins post-dose epoch [$t(216)=2.67$, $p<0.1$].

There was also a higher order interaction of treatment x epoch x consumer status [$F(12,216)=1.97$, $p<0.05$, $\eta_p^2=0.99$, observed power=0.91] (see Figure 2). The Bonferroni adjusted planned comparisons conducted separately within the habitual/non-habitual consumption status groups between caffeine and placebo revealed that whereas there were no significant reductions in CBF as indexed by total-Hb in the habitual consumers the non-habitual consumers showed a significant reduction during each cognitive task period epoch (31-34 min [$t(216)=4.24$, $p<0.01$], 35-39 min [$t(216)=3.39$, $p<0.05$], 40-43 min [$t(216)=5.47$, $p<0.01$], 44-48 min [$t(216)=4.35$, $p<0.01$], 49-52 min [$t(216)=6.00$, $p<0.01$], 53-57 min [$t(216)=5.50$, $p<0.01$], 58-61 min [$t(216)=7.13$, $p<0.01$], 62-66 min [$t(216)=3.55$, $p<0.05$]).

Figure 2 about here.

DISCUSSION

In the current study the administration of 75mg of caffeine led to decreases in CBF as indicated by concentration changes in total levels of haemoglobin in the prefrontal cortex. However, there was a significant interaction whereby this haemodynamic response to caffeine was dependent on the habitual consumption patterns of the participants, with no significant effects being shown in habitual caffeine consumers and an exaggerated effect being seen in non-habitual consumers of caffeine. This observation suggests that the effect of caffeine on local CBF is subject to tolerance as a consequence of chronic caffeine exposure. In this instance, modulation of cognitive performance was evident in terms of improved accuracy of the Serial Sevens task following caffeine, and no differences were seen in ratings of mental fatigue or task difficulty.

The interaction between consumption status and caffeine is in contradiction to previous suggestions that there is little evidence of tolerance to the effects of chronic caffeine consumption in terms of reduced CBF following consumption (Addicott et al., 2009; Mathew & Wilson, 1985) and that chronic caffeine consumption simply results in increased resting CBF in a state of caffeine withdrawal, with the magnitude of this effect correlated with everyday consumption (Field et al., 2003). Interestingly, several studies that have not assessed CBF but have focussed rather on related parameters have demonstrated similar interactions as seen here. For instance, Laurienti et al. (2002) assessed the effect of caffeine on the fMRI blood oxygen level dependent (BOLD) signal; a measure that represents the change in the proportions of oxyhaemoglobin and deoxyhaemoglobin, and which is taken as a proxy for neural activation. They demonstrated a significantly greater BOLD signal increase following administration of 250mg of caffeine to high caffeine consumers than to low consumers, and a significant correlation between changes in BOLD and habitual caffeine consumption. The authors propose that this difference is due to increased neural activation in high users as a result of upregulation

of adenosine receptors leading to a disruption in the 'normal state' ratio of A1:A2 receptor effects, which favours vascular reactivity. Similarly, Dager et al (1999), using proton echo-planar spectroscopic imaging found that in caffeine intolerant non-consumers, and in consumers withdrawn from caffeine for one month, but not in acutely withdrawn caffeine consumers, caffeine led to increases in global and region specific levels of lactate in the brain, potentially via the modulation of glycolysis by caffeine and reduced CBF.

In general, studies measuring parameters related to CBF following caffeine have taken measurements during a single, discrete post-dose period. Methodologies have included PET (Cameron, Modell, & Hariharan, 1990), Trans-cranial Doppler (Jones, Herning, Cadet, & Griffiths, 2000; Sigmon, Herning, Better, Cadet, & Griffiths, 2009) and MRI (Field, Laurienti, Yen, Burdette, & Moody, 2003, Addicott et al. 2009; Perthen). The current study utilised NIRS to confirm the previous observation of caffeine related reductions in CBF and this gave the added advantage of continuous recording from pre-dose until 66 minutes post-dose. As well as increasing the descriptive potential of the study, the use of multiple epochs (in this case data was collapsed into 5 minute epochs to capture the full dataset in the most parsimonious manner) also had the advantage of increasing the statistical power of the approach, and this may well have allowed for the detection of relatively subtle between group effects that might have eluded a single measurement.

It is notable in this regard that most studies assessing the effects of caffeine on CBF have also used a high (≥200mg) dose of caffeine that is approximately equivalent to the average UK daily dose (Brice & Smith, 2002). The current study used a dose of 75mg caffeine that was chosen on the basis that it is approximately equivalent to a single dose of caffeine (i.e. one cup of coffee) for an average consumer and is a typical dose employed in studies of the cognitive effects of caffeine. In this respect it has been shown to consistently engender psychoactive effects, including in non-consumers (Haskell et al. 2005). It is therefore possible that the

habituation of regular consumers seen here is only evident at lower doses, with 200mg+ being sufficient to obscure the effect and promote vasoconstriction across all individuals, irrespective of consumption habits. The assertion that habitual consumers might not demonstrate a significant vasoconstrictive response to caffeine at the 'everyday' levels administered here is supported by the findings of Chen and Parrish (2009b), who assessed the dose ranging effects of 1mg/kg, 2.5mg/kg and 5mg/kg of caffeine using fMRI in a cross section of withdrawn consumers. They demonstrated a linear dose response for CBF, but found no significant effects on either CBF or the BOLD signal following the 1mg/kg dose. This dose would be equivalent to the dose used in the current study for an average weight adult.

The haemodynamic effects of caffeine are due to interactions with both adenosine A_1 receptors, which are widely distributed throughout the brain and affect neuronal firing rate, and A_{2A} receptors, which are more prevalent throughout the cerebrovascular system where they modulate vasodilation (Laurienti et al., 2003). It is not clear whether the differences in response to withdrawal and/or administration of caffeine in relation to consumer status are the result of adaptations of the adenosine receptor system as a consequence of chronic caffeine consumption or are the result of underlying differences that actually drive consumer choice. Support for the assertion that genetic factors may impact upon caffeine consumption comes from twin studies reporting high heritability estimates for caffeine use, tolerance and withdrawal (Hettema, Corey, & Kendler, 1999; Kendler & Prescott, 1999; Luciano, Kirk, Heath, & Martin, 2005). This is further supported by genetic polymorphism studies showing specific genotypes that appear to be more prevalent in low caffeine consumers and are linked to increased sensitivity to the negative effects of caffeine (e.g. Alsene, Deckert, Sand, & de Wit, 2003; Cornelis, El-Sohemy, & Campos, 2007). However, a number of receptor binding studies have shown either upregulation of A_1 or A_{2A} receptors or an increase in affinity of either system, suggesting that adaptations to the adenosine receptor system do occur and an

upregulation/affinity of both A_1 and A_{2A} receptors could provide an explanation for the lack of cognitive deficits seen in caffeine withdrawal – any impairment as a result of reduced neuronal firing rate may be offset by the previously demonstrated increase in CBF (e.g. Field et al., 2003) resulting in increased supply of metabolic substrates. It is difficult to draw conclusions from these receptor binding studies as findings are far from consistent (Green & Stiles, 1986; Johansson, Georgiev, Lindstrom, & Fredholm, 1997; Shi & Daly, 1999; Varani et al., 2000; Varani et al., 2005; Varani et al., 1999), but adaptations of the adenosine receptor system as a consequence of chronic caffeine consumption may help to explain findings showing a neuroprotective effect of chronic caffeine use against the impact of ischaemic injury in rats (de Mendonca, Sebastiao, & Ribeiro, 2000; Li et al., 2008). The practice of ischaemic preconditioning involves brief periods of ischemia and reperfusion in order to build up resistance to subsequent ischemia, and the release of adenosine is pivotal to this (Riksen et al., 2006). Chronic caffeine consumption could be thought of as a subtle form of ischemic preconditioning carried out on a daily basis. However, acute caffeine consumption has been shown to prevent the effectiveness of ischaemic conditioning (Riksen et al., 2009) and previous studies of the acute pressor effects of caffeine have shown that only 50% of participants build up complete tolerance (Farag et al., 20005; Lovallo et al., 2004). The finding that caffeine consumption is only associated with an increased risk of myocardial infarction in those with the cytochrome P450 1A2 genotype (Cornelis et al., 2006) suggests that further work should be carried out with regards the involvement of specific genotypes in the impact of caffeine on cerebral haemodynamics.

In terms of the cognitive tasks, caffeine only resulted in improvements in the performance of the Serial Sevens task, with this evinced as greater accuracy. This reduction in errors as a consequence of consuming caffeine fits with the vast literature showing caffeine to be a cognitive enhancer. However, the tasks employed here were chosen on the basis that they have previously been shown to activate the prefrontal cortex (Drummond et al., 1999; Lawrence,

Ross, & Stein, 2002 Kazui et al., 2000). and engender a haemodynamic response using the same methods as employed here (Kennedy, Wightman et al., 2010), rather than on the basis of their sensitivity to caffeine. Relatively modest effects on performance were therefore expected. Although improvements in the performance of the RVIP task have previously been reported following caffeine this is not always the case, in particular when considering non-habitual caffeine consumers (Haskell et al., 2005; Smit & Rogers, 2000) or when the demands of the task are increased (Attwood, Higgs, & Terry, 2007). Caffeine administration generally has its most pronounced and consistent effects on mood, particularly subjective ratings of alertness. It is notable that these effects (and any modulation of 'headache' in consumers) were absent in the current study. However, this may simply reflect the masking of any such subjective effects by the unwieldy headgear and restricted movements imposed upon the participants. It is also noteworthy that these parameters were assessed prior to task performance, rather than after, when it is possible that participants would be more likely to be consciously aware of these effects. In terms of relating any CBF effects to behavioural effects, the findings from the current study suggest that these occur independently of each other, at least in terms of those aspects of behaviour studied here. This is true both of improvements in the performance of the Serial seven task that were seen irrespective of consumer status, and the lack of any other behavioural effects despite decreases in CBF. Interestingly, the study included a cohort of withdrawn caffeine consumers and a group that were not withdrawn due to their habitual low levels of caffeine consumption, but there were no differences between these consumption groups in terms of baseline cognitive performance or following placebo, or indeed following caffeine. These findings could be seen as arguing against the suggestion that any effects of caffeine on cognitive function are simply due to the alleviation of decrements due to caffeine withdrawal in experimental paradigms that tend to use abstinent caffeine consumers (see: James & Rogers, 2005). They rather offer some support to the opposite view, that caffeine engenders net effects

per se irrespective of withdrawal status (Haskell et al., 2005; Hewlett & Smith, 2006; Smith, Christopher, & Sutherland, 2006).

The current exploratory study makes two distinct contributions to research in this area. Firstly, it confirms the utility of NIRS as a tool for measuring the CBF effects of nutritional interventions, and, in this respect it complements the previous observation of a vaso-dilatory effect (i.e. the opposite to that seen here) following single doses of another plant chemical, the polyphenol resveratrol, using the same methodology (Kennedy, Wightman et al., 2010). It is interesting that potentially beneficial haemodynamic effects have been seen following treatment with a plethora of other food components, herbal extracts and food supplements, including (but not restricted to), Ginkgo biloba (Ahlemeyer & Krieglstein, 2003), tea (Alexopoulos, et al., 2008), tea catechins (Schroeter, et al., 2006) Epigallocatechin Gallate (EGCG) (Widlansky, et al., 2007), vitamins (Title, et al., 2000), and sources of dietary nitrate (Presley, et al., 2011). NIRS may well represent an effective and economical tool for extending investigations that have tended to concentrate on peripheral blood flow parameters to include the effects of these, and other, natural products on brain haemodynamics. Secondly, the observation of an interaction between caffeine consumption habits and its acute vaso-constricting effects, questions the utility of using caffeine as a 'contrast booster' in fMRI studies (Mulderink, et al., 2002), in particular when using a heterogeneous sample of participants in terms of caffeine consumption. They also suggest, along with the literature demonstrating increased resting basal CBF in withdrawn caffeine consumers, that issues revolving around the use of caffeine withdrawn or replete participants are more complex than previously anticipated, and that these complexities need to be considered in the design of any study that is predicated on measuring any parameters related to CBF, or indeed peripheral blood flow, in humans. In some ways this problem is liable to prove largely intractable, as the vast majority of the population (80 %+) consume caffeine on a daily basis.

Although the current investigation generated a clear pattern of results with regards CBF, several weaknesses and potential improvements have to be acknowledged. The first is that NIRS generates 'change in concentration' rather than quantitative data. This means that it is ideally suited to investigating the time course of the haemodynamic effects of any acute intervention continuously over a comparatively long period, provided recording is started pre-intervention in order to establish a treatment free baseline. However, the lack of quantitative data limits its utility in assessing haemodynamic responses in a chronic intervention context. We therefore could not assess the CBF effects of withdrawal in our two consumption groups. Naturally this information would have helped in the interpretation of the effects seen here, and in future, the addition of a quantitative technique such as TCD to add a measure of blood flow pre and post-withdrawal may have some utility. Similarly, as the task period started at 30 minutes post-dose and continued to the end of recording it is impossible to ascertain the relative contributions of the time course of caffeine's bioavailability and task performance *per se* to the effects seen here. The methodology could therefore be modified by including task performance/non-performance as a further factor in the design. It is also necessary to replicate this study with withdrawn and non-withdrawn conditions in order to assess the effects of caffeine administration in habitual consumers who are not in a state of withdrawal. It would also be useful to examine higher doses of caffeine to match the preponderance of other research in this area.

Whilst the current study employed a healthy sample size (N = 20) for a balanced cross-over, repeated-measures experiment, the subdivision of the sample by caffeine consumption status did lead to sub-optimal power for the consumption status effects. The sample size was also too small for a further meaningful sub-division into males and females in order to examine any gender differences in responses. Similarly, no account was taken of variations in the participants' sleep the night before testing (although testing took place at the same time for all participants), or the position of the female participants in their menstrual cycle. Whilst a larger

sample size would be preferable in future studies, and tighter control might be exercised in terms of sleep and hormonal fluctuations, it could also be argued that all of these factors could only have served to obscure a genuine effect, suggesting in turn that the interaction effects seen here should be comparatively robust.

The findings from the current study show that caffeine modulates cerebral blood flow. Although previous studies have suggested that these effects are not subject to tolerance as a consequence of chronic caffeine exposure the current findings suggest that tolerance to these effects may occur when considering a dose of caffeine equivalent to a normal single dietary serving. Previous studies may also have failed to find a difference between 'low' and 'high' consumers due to their 'low' consumers being in a state of withdrawal. Given the prevalence of caffeine consumption (91 % of a UK sample consumed daily caffeine amounts equivalent to at least one cup of tea – Heatherley, Mullings, Tidbury, & Rogers, 2006) it is essential that further work is carried out in order to fully understand the impact of caffeine and caffeine withdrawal. Given the low proportion of the population that are non-habitual caffeine consumers and the potential physiological differences between them and habitual consumers, research in this area should focus on long-term abstinent habitual caffeine consumers, covering a range of consumption levels and employing single doses equivalent to those consumed in our diet.

DISCLOSURE

The authors declare no conflicts of interest.

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Titles and legends to figures

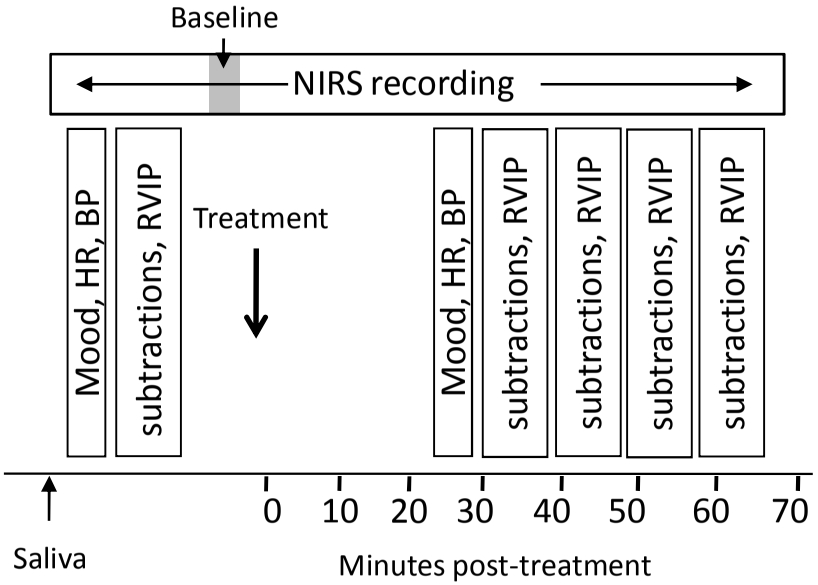
Figure 1. Timelines of each assessment. HR = heart rate; BP = blood pressure; RVIP = rapid visual information processing; NIRS = near infrared spectroscopy.

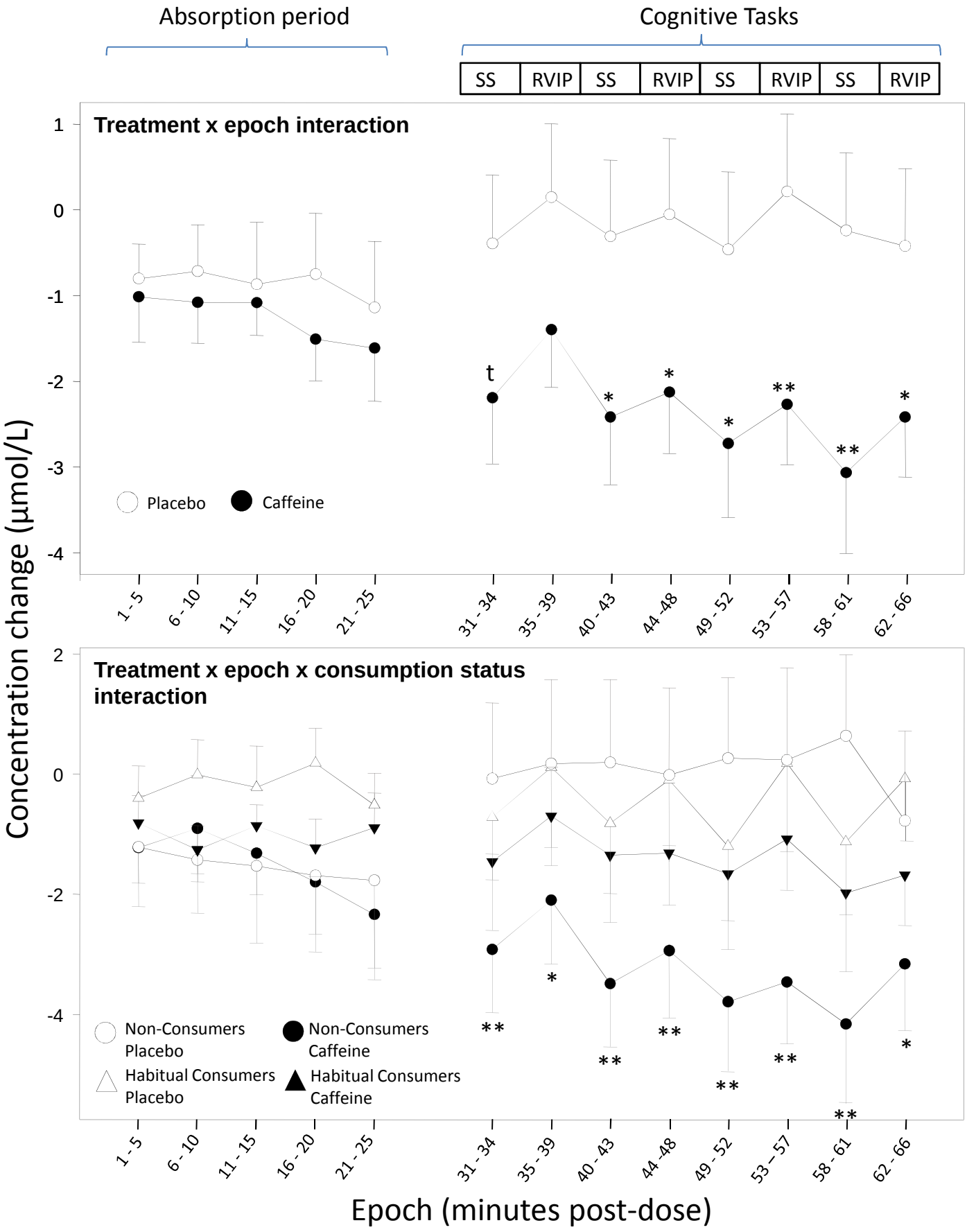
Figure 2. Cerebral blood flow as indexed by concentration changes in total levels of haemoglobin in the frontal cortex during a 25 minute absorption period and subsequent 36 minutes of cognitive task performance. Data are averaged across the 5 minute (absorption period, RVIP) or 4 minute (Serial Subtraction) epochs, with SEM error bars. The top panel shows the interaction between treatment and epoch irrespective of consumption status with asterisks showing significance between treatment groups. The bottom panel shows the higher order interaction between treatment, epoch and consumption status with asterisks denoting significant treatment related differences within the consumption status groups. In the bottom panel all of the significant comparisons are between treatments in the non-consumers (t = $p < 0.1$; * = $p < 0.05$; ** = $p < 0.01$ using Bonferroni adjusted t tests) SS = serial subtractions tasks, RVIP = Rapid Visual Information Processing task.

Table 1. Baseline and change from baseline CDB scores and ratings following placebo and caffeine for the 'non-habitual/habitual' consumer groups. Data are mean scores and *SD*. Rep=repetition of CDB

Outcome	Status	Treatment	N	Baseline		Rep 1		Rep 2		Rep 3		Rep 4	
Serial Threes Total (Number)	**Non-habitual**	*Placebo*	10	43.4	*15.3*	3.20	*5.63*	-1.70	*6.41*	0.80	*7.05*	0.40	*7.56*
		Caffeine	10	42.6	*15.1*	2.90	*6.51*	2.50	*5.15*	3.40	*6.83*	5.40	*8.26*
	Habitual Consumers	*Placebo*	9	42.2	*12.4*	1.78	*4.63*	5.56	*6.80*	6.22	*7.48*	6.11	*6.58*
		Caffeine	9	44.1	*9.00*	0.78	*5.91*	1.67	*6.58*	2.22	*7.60*	0.44	*8.71*
Serial Threes Errors (Number)	**Non-habitual**	*Placebo*	10	0.80	*1.23*	0.70	*1.25*	0.10	*1.20*	1.50	*1.43*	1.30	*2.00*
		Caffeine	10	2.10	*2.13*	-0.80	*1.93*	-0.50	*2.32*	-0.20	*2.70*	0.40	*2.07*
	Habitual Consumers	*Placebo*	9	1.44	*1.67*	0.40	*2.17*	0.70	*2.95*	1.70	*4.06*	0.78	*2.28*
		Caffeine	9	1.56	*1.59*	0.10	*1.52*	1.30	*2.16*	0.50	*2.42*	0.90	*3.00*
Serial Sevens Total (Number)	**Non-habitual**	*Placebo*	9	25.6	*9.45*	2.00	*2.55*	2.89	*3.10*	1.56	*6.89*	2.00	*5.55*
		Caffeine	9	26.4	*11.1*	-0.67	*4.47*	1.22	*1.99*	0.89	*4.68*	1.89	*5.42*
	Habitual Consumers	*Placebo*	10	24.3	*9.37*	-0.50	*3.66*	1.10	*6.44*	2.30	*6.43*	2.60	*6.24*
		Caffeine	10	23.5	*9.32*	2.10	*3.96*	2.60	*4.14*	1.80	*4.13*	3.30	*4.90*
Serial Sevens Errors (Number)	**Non-habitual**	*Placebo*	9	0.89	*1.17*	1.33	*2.45*	1.00	*1.66*	1.44	*1.13*	1.44	*1.33*
		Caffeine	9	2.00	*2.12*	0.22	*1.79*	-0.44	*2.07*	-0.22	*2.28*	-0.67	*1.94*
	Habitual Consumers	*Placebo*	10	1.70	*1.34*	1.50	*1.78*	0.90	*2.23*	0.50	*1.58*	1.30	*1.34*
		Caffeine	10	1.70	*1.57*	0.30	*1.77*	-0.20	*1.40*	0.10	*0.99*	0.30	*2.26*
RVIP Accuracy (%)	**Non-habitual**	*Placebo*	10	65.3	*19.1*	4.50	*7.15*	-6.25	*9.52*	-3.50	*10.2*	-5.00	*8.16*
		Caffeine	10	73.3	*14.3*	2.25	*7.95*	-8.00	*9.92*	-8.50	*10.8*	-9.25	*13.3*
	Habitual	*Placebo*	10	57.0	*24.8*	2.00	*6.85*	-5.50	*6.21*	-3.00	*9.63*	-3.25	*6.35*

	Consumers	*Caffeine*	10	53.0	*23.7*	4.25	*10.9*	1.50	*10.7*	-3.75	*11.9*	0.00	*13.5*
RVIP Reaction Time (msecs)	**Non-habitual**	*Placebo*	10	513	*37.7*	-16.3	*27.7*	-12.1	*31.5*	-15.0	*33.5*	-18.5	*24.7*
		Caffeine	10	488	*30.6*	-2.38	*28.2*	16.8	*33.7*	18.9	*29.3*	20.3	*36.5*
	Habitual Consumers	*Placebo*	10	505	*80.1*	28.3	*21.8*	5.60	*45.8*	5.55	*26.8*	1.75	*47.5*
		Caffeine	10	518	*73.9*	3.87	*36.2*	3.24	*44.3*	-10.1	*48.2*	-15.9	*41.9*
RVIP False Alarms (Number)	**Non-habitual**	*Placebo*	10	3.80	*2.53*	-1.20	*3.12*	-0.50	*3.14*	-1.30	*2.54*	0.30	*3.23*
		Caffeine	10	2.90	*2.85*	-0.90	*1.60*	0.70	*3.30*	-1.50	*2.17*	-0.10	*2.02*
	Habitual Consumers	*Placebo*	10	2.00	*1.76*	1.20	*3.58*	2.00	*4.92*	2.40	*6.92*	2.20	*6.27*
		Caffeine	10	3.50	*3.50*	0.30	*4.62*	0.00	*3.27*	-0.50	*3.34*	0.10	*4.07*
Mental Fatigue (mm)	**Non-habitual**	*Placebo*	10	52.3	*15.5*	-0.10	*16.5*	8.50	*11.7*	11.4	*14.6*	18.1	*12.8*
		Caffeine	10	41.3	*19.0*	6.60	*13.3*	12.9	*20.0*	14.9	*18.8*	12.9	*18.9*
	Habitual Consumers	*Placebo*	10	51.0	*16.9*	6.90	*16.4*	7.10	*19.1*	6.10	*26.9*	15.1	*20.0*
		Caffeine	10	48.5	*19.1*	5.30	*16.8*	9.00	*13.5*	13.6	*15.4*	18.6	*15.7*
Difficulty Rating (mm)	**Non-habitual**	*Placebo*	10	50.2	*15.3*	1.10	*8.90*	2.70	*14.0*	8.10	*14.4*	11.8	*16.1*
		Caffeine	10	37.9	*14.3*	9.60	*16.9*	7.60	*18.5*	16.9	*20.1*	16.7	*19.7*
	Habitual Consumers	*Placebo*	10	50.4	*13.7*	4.50	*9.74*	8.00	*9.61*	9.70	*12.8*	13.7	*13.6*
		Caffeine	10	46.7	*17.7*	7.80	*14.3*	10.0	*16.3*	13.3	*15.6*	10.8	*20.3*


Baseline
NIRS recording
Mood, HR, BP
subtractions, RVIP
Treatment
Mood, HR, BP
subtractions, RVIP
subtractions, RVIP
subtractions, RVIP
subtractions, RVIP
0
10
20
30
40
50
60
70
Saliva
Minutes post-treatment


Absorption period
Cognitive Tasks
SS
RVIP
SS
RVIP
SS
RVIP
SS
RVIP
Treatment x epoch interaction
Placebo
Caffeine
t
Treatment x epoch x consumption status interaction
Non-Consumers Placebo
Non-Consumers Caffeine
Habitual Consumers Placebo
Habitual Consumers Caffeine
Concentration change (µmol/L)
1 - 5
6 - 10
11 - 15
16 - 20
21 - 25
31 - 34
35 - 39
40 - 43
44 - 48
49 - 52
53 – 57
58 - 61
62 - 66
Epoch (minutes post-dose)