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Acute effects of brewed cocoa consumption on attention, motivation to perform cognitive work and feelings of anxiety, energy and fatigue: a randomized, placebo-controlled crossover experiment

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Abstract

Background: Acute effects of caffeinated and non-caffeinated cocoa on mood, motivation, and cognitive function are not well characterized. The current study examined the acute influence of brewed cocoa, alone and with supplemental caffeine, on attention, motivation to perform cognitive tasks and energy and fatigue mood states.

Methods: A randomized, double-blinded, within-subjects crossover trial was conducted with four 473-milliliter brewed beverage treatments: cocoa, caffeinated cocoa (70 milligrams caffeine total), placebo (flavored and colored brewed water) and positive control (placebo plus 66 milligrams caffeine, “caffeine alone”). Participants ($n = 24$) were low consumers of polyphenols without elevated feelings of energy. Before and three times after beverage consumption, a 26-minute battery was used to assess motivation to perform cognitive tasks, mood and attention (serial subtractions of 3 and 7, the continuous performance task, and the Bakan dual task) with a 10-minute break between each post-consumption battery. The procedure was repeated with each beverage for each participant at least 48 h apart and ± 30 min the same time of day. Data were evaluated using Treatment X Time analysis of covariance controlling for hours of prior night's sleep.

Results: Compared to placebo, cocoa reduced overall false alarm errors progressively across time with 0.92, 1.44 and 2.35 fewer false alarms on average 22–48, 60–86 and 98–124 min post-consumption ($\eta^2 = 0.08$, $p = 0.019$). Caffeinated cocoa: (i) attenuated the anxiety-provoking effects of cognitive testing found after drinking caffeine alone ($\eta^2 = 0.064$, $p = 0.038$), and (ii) increased accuracy ($\eta^2 = 0.085$, $p = 0.01$) and reduced omission errors ($\eta^2 = 0.077$, $p = 0.016$) on the Bakan primary task compared to cocoa alone.

Conclusions: Brewed cocoa can acutely reduce errors associated with attention in the absence of changes in either perceived motivation to perform cognitive tasks or feelings of energy and fatigue. Supplemental caffeine in brewed cocoa can enhance aspects of attention while brewed cocoa can attenuate the anxiety-provoking effects found from drinking caffeine alone.

Trial registration: ClinicalTrials.gov Identifier: NCT01651793. Registered July 25, 2012.

Keywords: Anxiety, Attention, Caffeine, Cocoa, Energy, Fatigue, Flavanols, Mood, Theobromine, Vigilance

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Background

Past researchers have examined the cardiovascular health effects of acute and chronic cocoa consumption [1, 2] and acute brain vascular changes after cocoa consumption also have been documented [3, 4]. However, the potential short-term effects of cocoa on mood, motivation and cognitive function are less well characterized.

To date, cocoa has been examined in forms containing other ingredients that can impact mental performance. For example, drinks containing caloric energy that increase blood glucose consistently improve performance on memory and attention tasks [5, 6]. Caffeine also has well documented attention, motivation and mood enhancing effects [7–9] and these effects may occur as quickly as 10 min (mins) post-consumption [10]. Cocoa contains a small amount of caffeine (approximately 5-fold and 20-fold less caffeine per ounce than cola and coffee, respectively), but even small amounts of caffeine can influence attention and mood [11, 12]. Despite the existence of commercially available cocoa products with added caffeine, investigations examining the psychological consequences of interactions between constituents in chocolate or cocoa-containing beverages are rare. Related studies, such as those examining glucose and caffeine or cocoa and theobromine, suggest possible synergistic effects on aspects of cognitive performance [13–15]. Conversely, there is inconsistent evidence from small studies showing that the consumption of cocoa with milk can reduce the bioavailability of flavanols [16]. If this is true then the potential effects of cocoa flavanols on mood and cognitive performance may be underestimated when cocoa is co-consumed with dairy products. Only one other study has examined cocoa in the absence of dairy or calories and it was found that the consumption of tablets containing 250 mg cocoa transiently improved self-reported mental fatigue and serial sevens performance compared to placebo [17].

Chocolate and cocoa-containing beverages, which are often made or consumed with milk, contain compounds, such as choline and tryptophan, that cross the blood-brain barrier and could influence mood, motivation or cognitive performance [18]. The potential effects of cocoa on mood and cognition also have been hypothesized to result from cocoa flavanols or the dominant methylxanthine contained in cocoa – theobromine [19].

There is a small but growing body of research on the cognitive and mood consequences of chocolate and cocoa consumption [17, 20–23]; however, there appear to be only a few studies concerning the influence of the consumption of cocoa flavanols *per se* on acute changes in cognitive performance or mood. One experiment found that, compared to white chocolate containing trace amounts of flavanols, the consumption of dark chocolate containing 773 milligrams (mg) of cocoa

flavanols improved spatial memory and reaction time during the predictable phase of an attention task performed 2 to 2.75 h (hrs) post-consumption [24]. Mood and motivation were not measured in that study, but motivation is a factor that could plausibly be influenced by cocoa and is known to impact tasks of attention [25]. A second experiment examined effects of two identical dairy-based drinks with doses of cocoa flavanols of either 520 or 994 mg on both mood and a cognitive performance test battery. The drink containing 520 mg of cocoa flavanols had the largest and most consistent psychological effects - increased performance accuracy during a test of attention and reduced ratings of mental fatigue from 1.5 to 2.5 h post-consumption [26]. A third experiment showed no effect of 100 mg, 200 mg or 300 mg theobromine delivered in a cocoa-based beverage on mood state or vigilance [27]. Hrs of sleep the night before testing was not considered in any of these studies despite strong evidence that variations in sleep can result in meaningful changes in mood and cognitive performance [28–30].

The aim of the present experiment was to examine the acute influence of brewed ground cocoa, both alone (no dairy, no calories) and with supplemental caffeine (49 mg added resulting in 70 mg total, an amount not exceeding the US Food and Drug Administration limit for cola drinks), on attention, motivation to perform cognitive tasks, and energy and fatigue mood states.

A second purpose was to determine if the mood, motivation, or cognitive effects occur sooner than 1.5 h after consumption. Prior studies used a 1.5 to 2.75 h post-consumption time frame because increases in cerebral blood flow were found 2–4 h post-consumption [4]. This brain blood flow study [4], however, did not examine any time periods less than 2 h post-consumption. The bioavailability of active ingredients in cocoa and the subsequent mood, motivation, and cognitive effects plausibly could occur more quickly when cocoa is consumed in the absence of dairy products as has been shown for antioxidant levels after consumption of chocolate with and without milk consumption [16].

The study hypotheses were that during tests of attention (i) brewed cocoa alone would quickly (i.e., in less than 2 h and in as little as 22 to 48 min post-consumption) improve performance on attention tasks, motivation to complete the cognitive tasks, and feelings of energy and fatigue, and (ii) that caffeinated brewed cocoa, compared to either brewed cocoa alone or caffeine alone, would result in improved attention, motivation, and feelings of energy and fatigue.

Methods

Design

A placebo-controlled, double-blinded, within subjects, randomized cross-over experiment examined the effects

of two brewed treatments, a positive control, and a placebo (each 473 milliliters; ml). The treatments were cocoa (21 mg caffeine, 179 mg theobromine, 499 mg flavanols and one packet Truvia sweetener) and cocoa + caffeine (70 mg caffeine, 179 mg theobromine, 499 mg flavanols and 1 packet Truvia sweetener). In order to better interpret potential null findings, a “caffeine-only” condition (473 ml brewed water containing 66 mg caffeine, caramel coloring and one packet Truvia sweetener) matched to the cocoa + caffeine condition was used to document whether the participants were responsive to a stimulus known to alter motivation, mood, and cognitive performance. The fourth condition was a placebo containing neither cocoa nor caffeine (473 ml of brewed water, caramel coloring and one packet Truvia sweetener). A mental energy test battery was administered before and three times after (22–48, 60–86 and 98–124 min) beverage consumption.

Screening

Potential participants were recruited from (i) large university classes, (ii) announcements on buses, bulletin boards, and electronic listservs, and (iii) through word of mouth. Potential participants were invited to complete screening questionnaires (medical history, diet, mood) administered online using Zoomerang ><http://www.zoomerang.com/><.

Potential participants were excluded with body mass index > 30 or who reported: (i) an allergy to cocoa, chocolate, or caffeine, (ii) any smoking, or (iii) above average feelings of energy (scores > 12) during the week prior to the screening using the vigor scale of the 30-item Profile of Mood States (POMS) questionnaire [31]. Potential participants were also excluded because of over-the-counter and prescription medication use (except for contraceptives) or high consumption of flavanols during the prior month (>39 total combined servings of cocoa, caffeine, fruits or vegetables high in flavanols) using medical history and diet questionnaires described previously [32, 33].

Participants

An *a priori* statistical power analysis showed that 24 participants would provide statistical power of 0.81 to detect a 2 Group x 4 Time interaction effect size of 0.65 given a *p*-value of 0.05 and assuming a correlation across the repeated measures on Time of 0.70. [34]. One female was excluded due to outlying data. Characteristics of the final sample (*n* = 23) are reported in Table 1.

The number of hrs of reported sleep the night before each of the four testing sessions did not significantly differ between conditions (*p* = 0.767) and all participants reported refraining from cocoa or caffeine consumption during the 24-hrs prior to each testing day.

Table 1 Participant characteristics

| | |
|---|---------------|
| Sex (males/females) | 17/6 |
| Age (years) | 20.25 ± 2.23 |
| Height (cm) | 168.28 ± 1.19 |
| Weight (kg) | 67.05 ± 14.87 |
| Body Mass Index (kg/m ²) | 23.26 ± 3.84 |
| Race | |
| White | 15 |
| Black | 6 |
| More than one race | 2 |
| Amount of sleep on a typical night in the past month (hrs) | 7.4 ± 1.1 |
| Consumption of high-flavanol foods or beverages during the past month | |
| Caffeinated drinks (servings) | 0.79 ± 2.25 |
| Cocoa (servings) | 2.88 ± 2.29 |
| Fruits (servings) | 4.13 ± 3.1 |
| Vegetables (servings) | 14.88 ± 6.53 |

Data are reported as means ± standard deviations where appropriate

Salivary caffeine, theobromine and paraxanthine levels

Saliva samples were obtained by passive drool using the SalivaBio collection system (Salimetrics, State College, PA, USA). Samples were collected at the start of each testing day in order to confirm compliance with the instructions to avoid cocoa- and caffeine-containing foods and beverages. Post-test session saliva samples were obtained to estimate the association between changes in selected methylxanthines and changes in mood and cognitive performance. The saliva samples were frozen at -80 °C. After all samples were collected, they were shipped overnight in coolers with dry ice to the Department of Laboratory Medicine, Children’s Hospital Boston. The samples were analyzed for theobromine, caffeine and paraxanthine with liquid chromatography–tandem mass spectrometry using previously described methods [35].

Mental energy test battery

Consistent with prior related research, the mental energy test battery was comprised of self-reported motivation (0–10) [7], mood measures (i.e., mental and physical energy and fatigue state scales [7, 36] and the POMS [31]) and computerized cognitive tasks of attention (i.e., Serial 3 and 7 subtraction tasks [26], Bakan and Continuous Performance Tasks [7]. The mood and motivation questionnaires were completed online using Zoomerang. This approach required the mental and physical energy and fatigue scales to be modified from usual (0 to 100) to a 0 to 10 format. The timing of the mental energy test battery is detailed in Table 2.

Table 2 Timing of the mental energy test battery

| Task | Approximate Times (minutes) |
|--|-----------------------------|
| Motivation to perform cognitive tasks | 0.5 |
| Likert scales of energy and fatigue | 1 |
| POMS fatigue and vigor scales | 2.5 |
| Serial subtraction of the number three | 2 |
| Serial subtraction of the number seven | 2 |
| Continuous performance task | 2 |
| Bakan task | 16 |

The total duration of the mental energy test battery was 26 min

All cognitive testing was performed in a seated position in a thermoneutral (23 ± 1 °C), sound-attenuated [~ 60 dB(A) below ambient] chamber with lighting at ~ 80 lux. Visual stimuli were presented that required a finger response. Participants used either the keyboard or a key pad (RB-530 key pad, Cedrus, San Pedro, CA, USA) to respond to information presented on a 20" computer monitor. The Continuous Performance Task and the Bakan test were scored using Cedrus Data Viewer. Due to software scoring limitations, two research assistants manually scored the subtraction tasks independently and discrepancies were resolved.

Test beverages

The participants consumed one of four 473 ml beverages on each testing day. The beverages were brewed in a coffee maker (Mr. Coffee model#BVMGEHX23, Keurig®, Cleveland, OH) to a temperature of ~ 167 °F, and then allowed to cool uncovered for 7–8 min in a 1500 ml Vanity Fair Insulair cup until the temperature reached ~ 140 °F prior to being consumed. Six cups of distilled water were filtered through the coffee maker with ~ 1474 grams (cocoa or placebo) to produce 473 ml of beverage. The drinks were prepared by a research assistant who was not otherwise involved in testing that day. The drink was brewed after the completion of questionnaires asking about sleep and the consumption of caffeine, cocoa, or medications in the last 24 h. Dark coloring (DDW The Colour House- product 034, Lot# 201205080070) was added to the beverages to provide a uniform color to aid in blinding. Participants also wore a nose clip during beverage consumption and a lid covered the cup while the beverage was being consumed. Participants consumed the beverage within 10 min of being served (before min 48 of the experiment as shown in Fig. 1).

Test products were manufactured and supplied by the Hershey Company in individually wrapped bags, coded with a two-digit number that identified the test beverage. These products were stored in a cool (~ 24 °F), dry environment in a light-impenetrable container prior to preparation. A chemical analysis, performed by the Hershey Company, is provided in Table 3.

Procedure

Approval for the study was granted by the University of Georgia Institutional Review Board (Study # 00000311).

Prior to all testing days the participants were advised to abstain from chocolate/cocoa, caffeine and alcohol consumption, and the use of all medications except for oral contraceptives for a minimum of 24 h prior to each testing day. Participants were also advised to get a typical amount of sleep.

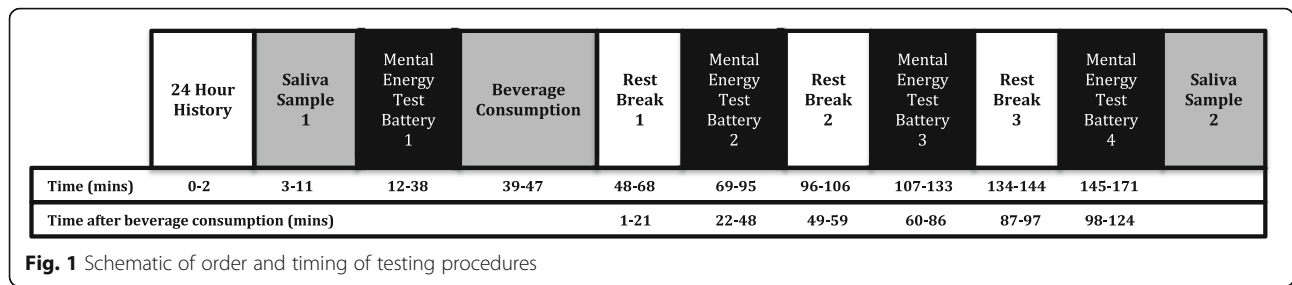
Familiarization Days 1–2. On Day 1, a 30–45 min single trial run of all daily assessments was conducted. On Day 2, the entire 2.75 h protocol was completed. Data from these familiarization days were not analyzed.

Testing Days 3–6: Four different treatment orders were used to minimize potential order effects. Participants were randomly allocated to complete one of four beverage orders (coded as 1-2-3-4, 2-3-4-1, 3-4-1-2 and 4-1-2-3) in blocks of four, such that each of the four orders was completed by six participants. With one exception there was a minimum of 48 h between testing days. Each participant was tested at the same time of day (± 30 min) to minimize potential diurnal variation. Because sleep loss has substantial effects on mood and cognitive performance [37], participants who reported 2 h more or less than their usual sleep duration (reported during the screening) were not tested that day and rescheduled, as were those who reported drug use or the consumption of cocoa or caffeine containing beverages or foods within the prior 24 h. The key testing events and their timing are presented in Fig. 1.

Data treatment and statistics

Preliminary analyses

Questionnaire data were downloaded into Excel from Zoomerang. Cognitive data were summarized using Cedrus Data Viewer (Cedrus Corp, 2007). All data were exported into SPSS (Version 20) for analysis. All statistical analyses were performed prior to breaking the blind. One individual had cognitive task performance scores that were deemed as error-dominated outliers (>3 standard deviations from the mean, invariant responding resulting in zero correct answers on multiple days, ID 54321). Data from this individual were excluded from the primary analysis. Scatterplots and descriptive statistics were evaluated. Variables that were not normally distributed (i.e., assessed from Kolmogorov-Smirnov tests, $p < .05$) were transformed using either a square root or log transformation prior to the primary analyses. The post-treatment minus pre-treatment changes in salivary concentrations of caffeine, theobromine and paraxanthine in the placebo, caffeine, cocoa and caffeinated cocoa conditions were examined using t-tests to examine whether the treatments influenced salivary methylxanthine concentrations in expected ways (e.g., caffeine increasing in caffeine



conditions; theobromine increasing in theobromine conditions).

Two participants (ID: 27051 & 34122) had baseline saliva samples on two of four testing days that contained >0.5 µg/ml caffeine and paraxanthine suggesting that they had failed to comply with the instructions to abstain from caffeine. When data from these participants were included, one-way ANOVAs revealed non-significant differences between the conditions in pre-testing salivary caffeine ($p = 0.50$) or paraxanthine ($p = 0.22$). Since the conclusions of the investigation were unchanged whether these participants were included or excluded, their data were included in the analysis. The conclusions of the investigation also were unchanged when the participants who used contraceptives were excluded.

Primary analyses

Hypotheses were tested using a series (i.e., all outcome variables) of two Treatment x 4 Time point, repeated measures ANCOVAs that controlled for the prior night’s sleep time. The primary interests were the presence of statistically significant ($p < 0.05$) interactions of time and either cocoa versus placebo, cocoa + caffeine versus cocoa, or cocoa + caffeine versus caffeine-only. Adjustments for sphericity, when needed, were made using Huynh-Feldt epsilon. Significant interactions were decomposed using one-way ANOVAs and t-tests with familywise error controlled using Least Significant Difference post-hoc tests. Effect size is presented as η^2 or Cohen’s d (calculated based on the mean change over time in a treatment condition minus the mean change over the same time in the placebo condition, and this difference score was divided by the baseline pooled

standard deviation). Cohen’s d values of .20, .50, and .80 are considered small, medium, and large effect sizes, respectively [38]. Pearson correlations (r) were used to explore linear associations between changes in salivary methylxanthines and changes in motivation, cognition, and mood.

Results

Expected changes in salivary methylxanthines were observed. Caffeine levels were increased significantly in the caffeine-only (mean change = 5.3 µmol·L⁻¹; $t = 8.676$, $df = 44$, $p < 0.001$) and cocoa + caffeine (mean = 5.0 µmol·L⁻¹; $t = 9.311$, $df = 44$, $p < 0.001$) conditions, and caffeine levels did not differ between these two conditions ($p > 0.50$). Theobromine levels were increased significantly in the cocoa (mean = 26.2 µmol·L⁻¹; $t = 11.655$, $df = 44$, $p < 0.001$) and cocoa + caffeine (mean = 28.9 µmol·L⁻¹; $t = 11.232$, $df = 44$, $p < 0.001$) conditions and theobromine levels did not differ between these two conditions. Paraxanthine levels were increased significantly in the caffeine-only (mean = 1.4 µmol·L⁻¹; $t = 2.689$, $df = 44$, $p = 0.01$) and cocoa + caffeine (mean = 1.1 µmol·L⁻¹; $t = 2.199$, $df = 44$, $p = 0.033$) conditions and paraxanthine levels did not differ between these two conditions. There were no statistically significant changes in all three methylxanthines in the placebo condition. Means and standard deviations for motivation, mood, and cognitive performance outcomes are available from the authors.

Effects of cocoa versus placebo

Compared to placebo, cocoa had significant interaction effects on both the reaction time response to the secondary targets on the Bakan test ($F = 2.679$, $df = 3, 129$, $\eta^2 = 0.071$, $p = 0.05$) and the overall false alarms on the Bakan test ($F = 3.735$, $df = 2.498, 107.42$, $\eta^2 = 0.08$, $p = 0.019$). Reaction times were faster at all post-test time points after consuming cocoa compared to pre-consumption baseline (range = 11–17 ms) while the comparable data after placebo were uniformly slower compared to baseline (range = 4–11 ms); the post-hoc tests were not statistically significant ($p > 0.05$). After taking cocoa the participants averaged 1.6 fewer false alarms compared to baseline while after placebo they averaged 2.4 more false alarms compared to baseline. At post-test time 3, the interaction

Table 3 Chemical analysis of the test beverages

| Beverage | ^a Total Flavanols (mg) | Theobromine (mg) | Caffeine (mg) |
|-------------------|-----------------------------------|------------------|---------------|
| Cocoa | 499 | 179 | 21 |
| Flavored placebo | 4 | 0 | 0 |
| Flavored caffeine | 4 | 0 | 66 |
| Caffeinated cocoa | 455 | 179 | 70 |

^aIncluding monomers, oligomers, and polymers

was significant ($t = 2.28$, $df = 44$, $p = 0.05$) and large ($d = 0.76$). No interactions were found for the other cognitive, mood and motivation variables.

Effects of cocoa + caffeine versus caffeine-only

Compared to caffeine-only, cocoa + caffeine had significant interaction effects on anxiety ($F = 2.963$, $df = 2.8$, 120.399 , $\eta^2 = 0.064$, $p = 0.038$). These data are illustrated in Fig. 2. At the final testing time anxiety levels increased by an average of 0.57 raw score units after caffeine alone but decreased by 0.17 raw score units after caffeinated cocoa. At the final testing time the effect size for the difference between conditions was large ($d = 0.84$) and statistically significant ($t = 2.27$, $df = 44$, $p = 0.028$). No significant interactions were found for all other mood, motivation and cognitive variables.

Effects of cocoa + caffeine versus cocoa

Compared to cocoa alone, cocoa + caffeine had significant interaction effects on the number of correct responses (i.e., accuracy) ($F = 3.971$, $df = 4.561$, 1.149 , $\eta^2 = 0.085$, $p = 0.01$) and the number of omission errors ($F = 3.583$, $df = 3$, 129 , $\eta^2 = 0.077$, $p = 0.016$) on the primary Bakan task. These interactions are illustrated in Fig. 3. The number of correct targets for the Bakan primary test steadily increased from baseline for cocoa + caffeine, whereas with cocoa alone the number correct was below baseline at post-test times 2 and 3 after a slight increase at post-test time 1. At the final testing time the effect size for the difference between the conditions in the number of correct responses was significant ($t = 2.45$, $df = 44$, $p = 0.0183$) and large ($d = 0.94$). Cocoa + caffeine also resulted in a steady decrease of the number of omission errors whereas cocoa alone led to increases. At the final testing time the size of the difference between the conditions in the number of omission errors was significant ($t = 2.14$, $df = 44$, $p = 0.0379$) and moderate

($d = 0.50$). No interactions were found for all other cognitive, motivation and mood variables.

Effects of caffeine-only versus placebo

No interactions were found for all cognitive, motivation and mood variables except for anger ($F = 4.419$, $df = 2.297$, 98.770 , $\eta^2 = 0.093$, $p = 0.011$). At the final testing time anger levels increased by an average of 0.66 raw score units after placebo, but were unchanged after caffeine-only. At the final testing time the size of the difference between the conditions was large and significant ($d = 1.07$; $t = 2.18$, $df = 44$, $p = 0.035$).

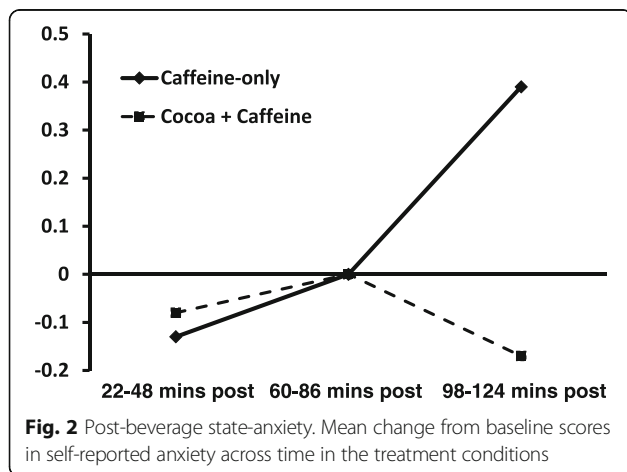
Relationships between changes in methylxanthines and changes in motivation, cognition and mood

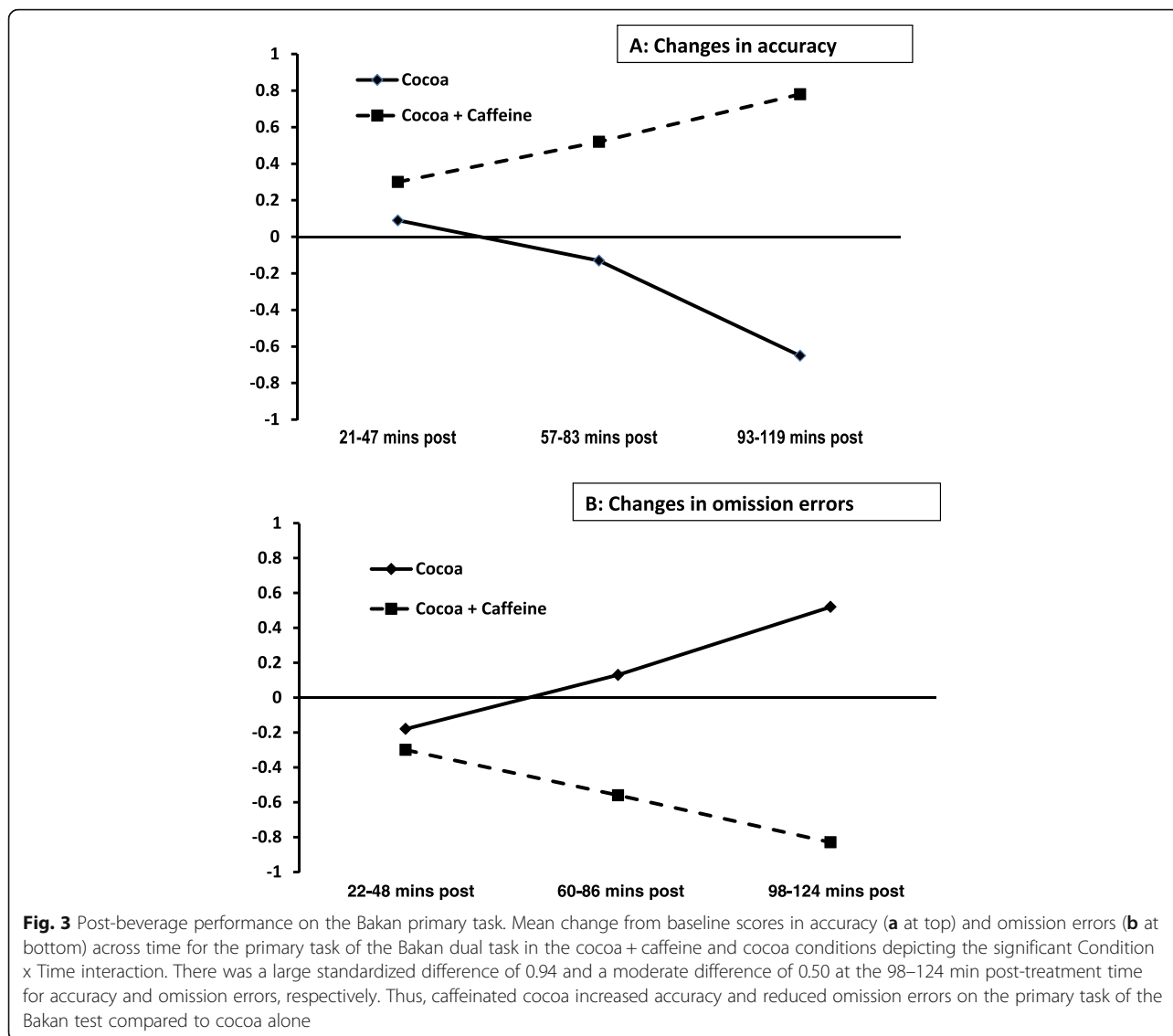
Changes in the methylxanthines were weakly and insignificantly related to changes in motivation, mood, and cognitive performance in all the treatment conditions except caffeine-only. In the caffeine-only condition, changes in salivary caffeine were significantly related to changes in physical fatigue ($r = 0.45$; $p = 0.031$) while changes in theobromine were positively correlated with changes in accuracy ($r = 0.51$; $p = 0.013$) and negatively correlated with changes in errors of omission ($r = -0.51$; $p = 0.013$) in the Bakan primary task. These relationships remained significant after partialling out correlated changes in caffeine ($r_{partial} = 0.50$ and $r_{partial} = -0.50$; both $p = 0.018$). Changes in paraxanthine were positively correlated with changes in accuracy ($r = 0.43$; $p = 0.041$) and negatively correlated with changes in errors of omission ($r = -0.43$; $p = 0.041$) in the Bakan secondary task. These relationships strengthened after partialling out correlated changes in caffeine ($r_{partial} = 0.58$; $p = 0.005$ and $r_{partial} = -0.56$; $p = 0.007$).

Discussion

Cocoa versus placebo

Cocoa enhanced two aspects of Bakan dual task performance compared to placebo. Cocoa reduced overall false alarm errors progressively across time with 0.92, 1.44 and 2.35 fewer false alarms on average at 22–48, 60–86 and 98–124 min post-consumption. Cocoa also improved processing speed during the secondary task of the Bakan dual task. The improvement in reaction time (11 ms faster) was apparent at 22–48 min post-consumption and there was a slight additional improvement (a total of 17 ms faster) that was maintained throughout the subsequent two testing times. Regression to the mean could not be ruled as an explanation for the significant effects of cocoa on the Bakan test because there were significantly fewer false alarm errors (mean = 4.6) and slower reaction time (mean = 25 ms) at baseline in the placebo condition compared to the cocoa condition. Mood states (i.e., POMS) were not improved after





taking cocoa alone compared to placebo which is consistent with studies that found no effect of theobromine on mood [14], but inconsistent with prior work suggesting that higher feelings of energy can increase performance in the high-event rate component of a dual task [39].

It is difficult to compare the Bakan secondary task results directly to other cocoa investigations because dual tasks were not used in the prior related cocoa studies [24, 26]. One prior study did not show fewer false alarms after 520- or 994-mg cocoa [26]. The failure of cocoa to significantly improve reaction time on the primary task of the Bakan test, serial three accuracy, serial seven errors, and feelings of mental fatigue were in contrast to the results of the study by Scholey and colleagues that is most similar in design to the present study [26]. A key difference between the present study and the Scholey study is the absence of dairy and calories in the present

study compared to the dairy-based cocoa drink with ~217 kcals used by Scholey and colleagues. The Bakan test used in this study also may have different psychometric properties from the conceptually similar rapid visual information processing test used in the Scholey et al. [26] study which may have contributed to different results. For example, the reliability or the sensitivity for measuring change might differ between the Bakan and the rapid visual information processing test because of procedural differences in the tests. The rapid visual information processing test requires participants to react to both odd and even sequences while the Bakan requires responses to odd sequences as a primary task and a single even number as a secondary task. Also, the Bakan task duration was three times longer and the stimuli in the rapid visual information processing test were presented at a rate of 100 per minute while the Bakan test presented

stimuli at a rate of 60 per minute. Another study using a 500-mg cocoa drink showed results that appear to be generally consistent with the present findings, but two of three testing times were confounded by the post-cocoa consumption of a lunch [40], which reduces the ability to make meaningful comparisons to the calorie-free cocoa drink used here.

Cocoa + caffeine versus caffeine-only

Cocoa + caffeine compared to caffeine-only allowed for an assessment of the potential role of cocoa flavanols combined with theobromine, which were both absent in the caffeine-only drink. Anxiety was the only significant interaction observed. Cocoa + caffeine attenuated the increase in anxiety that occurred at the final testing time in the caffeine-only condition. Elevated anxiety is a common side effect of caffeine consumption in low caffeine consumers [41] (such as those in this study) and many participants in past studies using similar protocols have anecdotally reported that repeatedly completing the attention task is stressful [7, 42]. Thus, the anxiety elevation at the final testing time in the placebo condition, while not hypothesized, is not unexpected. Theobromine and flavanols, or their metabolites, could plausibly influence anxiety by binding to adenosine or benzodiazepine receptors [42–44]. One study found that 500 mg cocoa acutely increased calmness; however, increased calmness did not occur after an acute cocoa administration at the start of the investigation but only after an acute administration was preceded by 30-days of daily cocoa supplementation [40], as could plausibly occur because of receptor up-regulation [45].

Cocoa + caffeine compared to cocoa

Cocoa + Caffeine compared to cocoa allowed for an assessment of the impact of 49 mg of supplemental caffeine on the outcomes. Supplemental caffeine improved accuracy and resulted in fewer omission errors on the primary task of the Bakan test, but otherwise had no statistically significant motivation, mood or cognitive interaction effects. Improved accuracy and fewer omission errors on the primary Bakan task occurred after the caffeine alone condition but the effect was smaller. Caffeine can improve vigilance performance by improving accuracy, reducing errors and reducing reaction time [46, 47] so it is unclear why the effects of supplemental caffeine were limited to the primary task of the Bakan test. One possibility is that the participants in the present study were not especially responsive to the mood, motivation and attention enhancing influence of caffeine. Genetic factors are known to influence caffeine sensitivity and relevant genotypes, such as for adenosine A_{2A} receptors, were not assessed in this study [42]. Another possibility is that caffeine may only influence the most challenging

component of the more difficult dual task. It has been suggested that while high event tasks take more cognitive resources, low event tasks, such as the primary task of the Bakan, require greater vigilance [48].

Caffeine-only versus placebo

Caffeine alone resulted in small changes that were generally in the direction expected based on prior research [49] but were small in magnitude and statistically non-significant. For instance, compared to pre-test, there were small, non-significant increases in motivation, feelings of energy and accuracy in the cognitive tests as well as small decreases in fatigue, errors and reaction times. Mean anger scores did not change in the caffeine condition, as is consistent with prior studies [50]; however, a significant interaction emerged because anger increased in the placebo condition. We speculate that anger scores increased in response to the stress of completing 104 total mins (4 x 26 mins sessions) of sustained vigilance testing across 2.75 h testing sessions and caffeine attenuated the effect.

Possible mechanisms

Caffeine crosses the blood-brain barrier and exerts central nervous system (CNS) effects by antagonizing adenosine receptors [51]. Dietary flavonoids are less well studied but experiments in rodents and pigs show that polyphenols can traverse the blood-brain-barrier and accumulate throughout the brain [52] and act on neural or glial cell-signaling pathways and increase cerebral blood flow [53]. One human study showed increased cerebral blood flow 2–4 h after consuming cocoa flavanols and a subsequent study found a similar increase in elderly persons, except that it was delayed until 8 h after ingestion [4, 54]. Thus, it is possible that the cognitive effects observed in the present study were the result of changes in brain blood flow, although no study has measured such responses < 2 h after cocoa administration. Adequate brain blood flow is known to be required for normal cognitive performance [55] but nutrition-induced increases in blood flow do not always produce improvements in cognitive performance [56]. Adequate blood flow to cognition-related neural circuitry is necessary but cognitive performance also appears to depend on a host of excitatory and inhibitory neurotransmitters (e.g., gamma-aminobutyric acid and glutamate), neuromodulators (e.g., dopamine and norepinephrine) and neuropeptides (e.g., cholecystokinin, corticotropin releasing factor, galanin) [57]. For example, caffeine can reduce overall and regional brain blood flow [58, 59] yet cognitive performance is often improved after caffeine is consumed. Therefore, it is plausible that the effects observed in the present study were not exclusively explained by blood flow changes.

Brain neurons use glucose for energy and the treatment effects observed here could stem from actions on glucose or its regulation [6]. Both caffeine and dietary flavonoids can impair glucose regulation [60, 61]; consequently, improvements in blood flow may have been opposed by alterations in glucose regulation. Also, the methylxanthine treatments may have stimulated the release of neurotransmitters or neuromodulators. Increased dopamine release in the frontal, prefrontal and medial cortices is hypothesized to deactivate the default mode network and is known to play a role in attentional processing [62, 63]. It is thought that caffeine antagonizes adenosine receptors in the basal ganglia which is known to contribute to the modulation of the default mode network [63, 64]. Increased dopamine in the nucleus accumbens also plays a role in motivation and feelings of energy [65]. One study comparing the mood and cognitive effects of theobromine and caffeine concluded that theobromine might exert anti-anxiety effects by lowering blood pressure rather than directly influencing the CNS. In short, the methylxanthines studied here potentially work via multiple, complex, interacting central and peripheral mechanisms. The present study was not designed to obtain data directly related to any of these potential mechanisms.

This study did obtain correlational data that could, indirectly, have relevance for the mechanisms involved in the behavioral effects observed here. In the caffeine only condition, changes in theobromine and paraxanthine were positively related to changes in accuracy and negatively related to changes in omission errors, but only in the more difficult Bakan dual task. These associations were attenuated when caffeine was combined with cocoa or when cocoa was consumed alone. The overall pattern of results suggests changes in cognitive performance and changes in salivary methylxanthine metabolites measured 2-hrs after 66-mg caffeine consumption are only modestly related, task dependent, and attenuated by the co-consumption of cocoa.

The correlational finding related to mood suggests that participants with higher salivary caffeine 2-hrs post-consumption, and hence with a slower metabolism of caffeine, also showed a greater increase in feelings of physical fatigue 2 h after caffeine had been consumed. It is uncertain why a correlation of a similar magnitude did not emerge for mental fatigue also measured with a visual analog scale ($r = 0.12$) or fatigue measured with the POMS category scale ($r = 0.26$). It should be noted that physical activity is not required to induce feelings of physical fatigue. Indeed, recent studies show that sitting and being sedentary for extended periods can contribute to feelings of fatigue [66]. This effect may be exacerbated by cognitive work involving attention.

Limitations

The study reported here had several features that may limit the generalizability of the findings. First, recruitment was limited to those reporting average or lower than average consumption of fruits and vegetables and other foods and beverages containing flavanols. Second, not all participants were medication-free, a relatively small number of participants were tested, and the timing and composition of the meals preceding testing were not controlled. Third, the potential role of sensory aspects of cocoa were not examined; there is evidence that sensory aspects of another drink made from cacao beans (e.g., mouth exposure to chocolate milk) can produce specific brain responses (e.g., increased blood flow in the orbitofrontal region) which may have contributed to changes in attentional task performance that were more rapid than any that stemmed from drink consumption [67, 68]. Fourth, we did not obtain saliva samples between completion of beverage consumption and the second mental energy test battery, so it is unclear if caffeine and metabolites were bioavailable prior to initiating the second mental energy test battery; however, previous evidence suggests the amount of time that orally consumed caffeine takes to reach peak bioavailability was within the time-range of the second mental energy test battery [69]. In addition, the cocoa or caffeine dose was not administered relative to bodyweight, but was absolute (i.e., 70 mg caffeine), which limits direct comparison to studies that did administer caffeine relative to body weight. Finally, the study design was block randomized (not fully randomized) and multiple statistical tests were conducted which increases the risk that one of the statistically significant results occurred by chance.

Conclusions

After statistically controlling for variation in the prior night's sleep duration, dairy- and calorie-free brewed cocoa can acutely influence aspects of attention but has little effect on motivation to perform cognitive tasks or mood states such as feelings of energy and fatigue. The caffeine in caffeinated cocoa can enhance attention while the brewed cocoa can attenuate the anxiety provoking effects of caffeine alone. The mechanisms by which these effects were caused remain to be elucidated.

Abbreviations

ANCOVA: Analysis of covariance; ANOVA: Analysis of variance; C: Centigrade; CNS: Central nervous system; dB (A): Decibels of sound pressure; hrs: Hours mg, milligrams; mins: Minutes; ml: Milliliters; ms: Milliseconds; POMS: Profile of mood states; SD: Standard deviation

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Availability of data and materials

All non-identifying data for this manuscript are available upon request to the senior author at poconnor@uga.edu.

Authors' contributions

PJO and SC conceptualized the study design. AB, JBL and BDL participated in collection of data. PJO and AB analyzed and interpreted the data and wrote the manuscript with comments from JBL, BDL and SC. PJO and JBL formatted the manuscript for submission. All authors read and approved of the final manuscript.

Competing interests

SC is a paid contractor for The Hershey Company.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Approval for the study was granted by the University of Georgia Institutional Review Board (Study # 00000311). All participants read and signed the approved consent form.

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