Impact of Various Caffeine Vehicles on Mood and Cognitive, Neurpological and Physiological Functions Over Five Hours

RYAN PAULUS[1], ALEX ROTH, LAUREN TITUS, RENEE CHEN, MICHAEL CHAD BRIDGES and SHYLA WOODYARD, College of Pharmacy, Ohio Northern University, Ada, OH USA

ABSTRACT. Although college students' caffeine consumption has increased over the last decade, studies have not yet determined the time frame in which caffeine exerts its effects nor the impact of the vehicle by which caffeine is consumed. Sixty college students were randomly divided into one placebo (flour) and three caffeine treatment groups: 5-Hour Energy®, Starbucks DoubleShot®, or caffeine powder. A battery of tests was performed prior to dosing and repeated 2.5 and five hours post treatment. Mood was self-reported on a scale of 1-100 for happiness, alertness and focus. Cognitive function was assessed by Stroop and memory tests. Reaction time, heart rate, blood glucose, and electroencephalogram were recorded. All initial measurements across groups and group baselines compared to 2.5 and 5 hour results were analyzed by ANOVA followed, when indicated, by post hoc t-tests at 95 percent confidence levels and only significant results are reported. All caffeine groups had elevations in mood and faster reaction times at 2.5 hours and most effects sustained for five hours. The 5-Hour Energy® group rated alertness higher than other caffeine treatments, and was the only group to demonstrate decreases in alpha waves, memory improvements, and impaired glucose homeostasis. All caffeine groups had improved cognition with decreased Stroop test time and the caffeine powder and 5-Hour Energy® groups had improved Stroop test accuracy at 2.5 hours. The 5-Hour Energy® shot had the greatest proportion of sustained caffeine effects across test parameters.

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INTRODUCTION

Over the last 10 years, college students have considerably increased caffeine consumption in delivery forms such as coffee and energy drinks (Bello 2008; McIlvain et al. 2011). The National Library of Medicine recommends only 200-300 mg of caffeine per day, but Nawrot et al. (2003) suggested daily doses up to 400 mg are not associated with adverse effects. A survey of college students conducted by Malinauskas et al. (2007), showed 51 percent of participants (n=253) reported consuming more than one energy drink each month with the majority of students using the drinks for insufficient sleep (67 percent) and to increase energy (65 percent). College students are not only relying on caffeine, but may be taking it at dangerous levels. In a 2011 survey of college students (n=300), 83 percent reported having had at least one sign/symptom of caffeine intoxication, 51 percent reported having at least one sign/symptom of caffeine withdrawal, and students consumed three to five times the recommended amount of caffeine (McIlvain et al. 2011).

Caffeine (1,3,7-trimethylxanthine) is a natural alkaloid found in coffee beans, tea leaves, cocoa beans, and cola nuts and has become the most commonly used behaviorally-active substance in the world (Giles et al. 2012). Ninety-nine percent is absorbed into the blood stream within 45 minutes of consumption with peak plasma levels within 15 - 120 minutes and a half-life between 2.5 - 4.5 hours (Smith 2002; Nehlig 2010). The hydrophobic properties of caffeine allow it to pass through the blood-brain barrier (Nehlig 2010) and bind to the adenosine receptors in the brain, acting as a competitive antagonist to adenosine (Poltev et al. 2010). Adenosine is involved in preparing the body for sleep by decreasing the communication between nerve cells and increasing the flow of oxygen by dilating the blood vessels. Presumably, by inhibiting adenosine, caffeine increases neuronal communication. Caffeine has consistently been shown to decrease EEG alpha waves (8 to 13 Hz) that appear during relaxation (Dimpfel et al. 1993; Gilbert 2000; Siepmann and Kirch 2002; Keane et al. 2007; Barry et al. 2008, 2009, 2011; Foxe et al. 2012). However, the effect of caffeine on beta waves (13 to 30 Hz), which appear with mental focus, is less consistent. Some researchers reported an increase in beta waves, (Patat et al. 2000; Keane et al. 2008; Sigmon et al. 2009) while others found a decrease (Dimpfel, et al. 1993; Gilbert, 2000; Siepmann and Kirch 2002; Barry et al. 2009). Along with a decrease in alpha waves, critical thinking used in

[1]Address correspondence to Ryan Paulus, 7865 Fleetfoot Road, Celina, OH 45822. E-mail: rpaulus2017@gmail.com

test taking requires the subject to interpret conflicting information. This conflict resolution occurs in the anterior cingulate cortex and the prefrontal cortex, and can be examined through a Stroop test, which assesses the ability to name a color word written in a conflicting color. Time to complete a Stroop test has been shown to decrease (Patat et al. 2000; Dixit, 2012; Pilli et al. 2013) or not change (Bottoms et al. 2013) after caffeine consumption.

Caffeine induces changes in physiological levels of neurotransmitters such as dopamine, adrenaline, serotonin, and acetylcholine (Dixit et al. 2012), which have been associated with alteration in attention, mood and physiological functions. According to Glade (2010), caffeine has been shown to: enhance cognitive performance, increase alertness, reduce reaction time, increase the ability to concentrate and focus attention, and enhance short-term memory. Additionally, caffeine is credited with decreasing fatigue (Loke 1988; Smit et al. 2004; Haskell et al. 2005; Arciero and Ormsbee 2009), increasing happiness (Smit et al. 2004; Arciero and Ormsbee. 2009), increasing alertness (Smit et al. 2004; Haskell et al. 2005; Rogers2008), and increasing vigor (Arciero and Ormsbee 2009; Souissi et al. 2013, 2012). Caffeine has also been suggested to improve reaction time (Haskell et al. 2005; Adan and Serra-Grabulosa, 2010; Souissi et al. 2013, 2012 et al. 2013; Santos et al. 2014). Other physiological effects of caffeine are not as clear cut. Caffeine has inconsistently been shown to increase heart rate (Alford et al. 2001), decrease heart rate (Arciero and Ormsbee 2009), or have no effect on heart rate (Bichler et al. 2006; Giles et al. 2012). Additionally, caffeine has been shown to increase blood glucose by impairing insulin sensitivity (Young and Benton 2013; Beaudoin et al. 2013; Cooper et al. 2014) or to have no effect on glucose levels (Hätönen et al. 2012).

While extensive research has shown the ability of caffeine to increase arousal and improve mood, attention and reaction time, tests of caffeine effects on memory have been inconclusive (Loke 1988; Smith 2002; Nehlig 2010). Auditory recall has been reported to be increased (Young and Benton 2013) or unaffected (Terry 1986; Sünram-Lea et al. 2012) by caffeine administration, and visual recall has been reported to be increased (Wesnes et al. 2013). Nehlig (2010) suggested that caffeine does not significantly influence intentional learning and memory tasks, but may facilitate memory during passive learning.

The majority of studies have credited caffeine as being the main enhancer of cognitive function in drinks containing multiple ingredients (Smit et al. 2004; Giles et al. 2012; Young and Benton, 2013). However, Young and Benton (2013) looked at the 150-minute time period post consumption of energy drinks composed of other ingredients, with or without caffeine, and determined that the long-term caffeine effect was altered by the drink with which it was consumed. Thus, the present study investigated differences in the vehicle in which caffeine was delivered, comparing caffeine powder capsules, 5-Hour Energy®, and Starbucks DoubleShot®.

In addition to caffeine, 5-Hour Energy ® contains B Complex vitamins and an energy blend containing citicoline and amino acids (Table 1). Thus, many of the 5-Hour Energy ® effects may be due to other ingredients or synergistic actions between ingredients. B Complex vitamins function as co-enzymes and precursors of cofactors in numerous enzymatic processes (Kennedy and Haskell 2011), however, the relationship between B vitamins and caffeine has not been studied (Childs, 2014). Short-term studies (one day to nine weeks) have suggested improved attention, processing skills, working memory, and subjective energy in young adults taking B vitamin supplements (Bryan et al. 2002; Kennedy et al. 2008; Haskell et al. 2010).

Citicoline promotes synthesis and transmission of neurotransmitters important to memory (McDaniel et al. 2003). Although there is evidence to support citicoline supplementation slowing cognitive decline in the elderly (Babb et al. 2002; García-Cobos et al. 2010), neither its interaction with caffeine nor behavioral effects in young adults have been extensively researched (Childs 2014). Amino acids such as taurine, tyrosine, and phenylalanine, are commonly added to energy drinks because they are precursors for neurotransmitters and are thought to increase neurotransmitter synthesis (Childs 2014). Most taurine studies have been with animals, but two human studies suggest that taurine counteracts the effects of caffeine on mood (Peacock et al. 2013; Giles et al. 2012). Tyrosine counteracts adverse effects from stress and fatigue by increasing attention, mood, and memory (Magill et al. 2003; Mahoney et al. 2007; O'Brien et al. 2007).

The major components of Starbucks DoubleShot® are caffeine and glucose (Table 1). Short-term studies (30 minutes post caffeine/glucose consumption) showed some degree of a synergism between caffeine

and glucose: increasing attention and memory (Scholey and Kennedy 2004), increased efficiency of attention system (Serra-Grabulosa et al. 2010), and improved reaction times (Adan and Serra-Grabulosa 2010). However, Smit et al. (2004) did not find significant synergy between glucose and caffeine. Young and Benton (2013) reported that glucose counteracted caffeine's effect on increasing subjective energy 30 minutes post consumption and slowed reaction times at 90 and 150 minutes. In addition, Giles et al. (2012) suggest that caffeine combined with glucose slowed reaction times. The inconsistencies in findings related to caffeine-glucose interactions were summed up by Mclellan and Lieberman (2012) as inconsistent due to limited quality experimental evidence to indicate that the addition of glucose to caffeine will cause greater improvements in physical and cognitive performance.

TABLE 1
Ingredients in 5-Hour Energy® and Starbucks DoubleShot®

Ingredient	Total Amt in Drink	Amt Given to 75 kg Individual
Starbucks DoubleShot®		
Total Fat	6 g	10.38 g
Saturated Fat	3.5 g	6.058 g
Cholesterol	20 mg	34.615 mg
Sodium	70 mg	112.15 mg
Total Carb	18 g	31.153 g
Sugars	17 g	29.422 g
Protein	4 g	6.923 g
Caffeine	130 mg	225 mg
5-Hour Energy®		
Niacin	30 mg	33.75 mg
Vitamin B6	40 mg	45 mg
Folic Acid	400 mcg	450 mcg
Vitamin B12	50 mg	562.5 mcg
Sodium	18 mg	20.25 mg
Caffeine	200 mg	225 mg
Energy Blend	1870 mg	2103.8 mg

Although many vehicles, particularly 5-Hour Energy®, claim a prolonged effect of caffeine consumption, most studies focused on the period 30-60 minutes post consumption (Young and Benton 2013). Wesnes et al. (2013) reported significant improvements in self-rated alertness, short- and long-term memory, focus, concentration and information processing following consumption of 5-Hour Energy® when compared to a placebo, and many of these effects were sustained up to six hours.

Students across college campuses consume caffeine to stay alert and study for long hours. However, there is a possibility that 2.5 to five hours post-consumption, caffeine's effect may be start to wear off. Many of the inconsistencies found with the effects of caffeine can be attributed to research methodology, including but not limited to, differences in the vehicle by which caffeine is consumed. Childs (2014) states that more research needs be done on the use of energy products by the people most apt to consume them, (adolescents and younger adults) and that there is a lack of empirical evidence for a beneficial reaction between caffeine and other ingredients.

Extensive research into the preferred vehicle for caffeine consumption has not been conducted, and Young and Benton, (2013) stressed the need for research on the efficacy of caffeine 90 minutes post-consumption. Therefore, questions arise on the long-term efficacy of caffeine and if the vehicle which caffeine is consumed effects the duration or efficacy of caffeine's effect. This study examined the differences in mood, cognitive, physiological and neurological effects of pure caffeine powder, 5-Hour Energy®, and Starbucks DoubleShot® vs. a placebo (flour capsule) for five hours post consumption among college students. It is hypothesized that the 5-Hour Energy® with B vitamins and an energy blend will effectively increase both the degree and duration of caffeine effects across test parameters.

METHODS

Participants

College students, divided between males and females ages 18-22 (n=60) were equally divided into four treatment groups (n=15) based on caffeine consumption. In this IRB approved study participants were screened to exclude heart conditions, gluten allergy, or color-blindness and were required to fast overnight (minimum of 12 hours). Testing was performed in a quiet University laboratory with no interfering noise or distraction.

Design and Treatment

Participants performed baseline-testing including: mood survey, auditory recall, visual recall, reaction test, heart rate, electroencephalograph (EEG), Stroop test, and blood glucose. Each caffeine delivery method was individually dosed to administer three mg of caffeine per kg of body weight (Dixit, 2012); thus, a 75 kg participant received 225 mg of caffeine powder, 67.5 mL of 5-Hour Energy® (5HE) (200 mg caffeine/60 mL), or 332.3 mL of Starbucks DoubleShot® (SDS) (130 mg caffeine/192 mL). Participants then consumed either a placebo (flour), caffeine powder in a capsule (CP), SDS, or 5HE and a whole-wheat bagel with cream cheese. Participants allowed to leave the laboratory after testing and were instructed to return 2.5 and five hours later, at which time the test battery was repeated. Participants were not allowed to eat any food or drink any beverage other than water. There were no restrictions placed on the amount of activity between testing periods.

Test Battery

Mood: Participants self-reported their mood using visual analogue scales (Young et al. 2013). At the ends of a 100-mm line were pairs of opposite adjectives that reflected the variables used in the Profile of Mood States Questionnaire: Happy/Sad, Relaxed/Stressed, Focused/Distracted, and Alert/Tired. Participants marked where their current mood fit along the scale.

Auditory Recall: A series of 20 words were broadcasted from a recording device with a word frequency of one word every 2.5 seconds. After completion of the recording, participants were instructed to write down as many words as they remembered (Sünram-Lea et al. 2012). A different word list was used at each testing period. Words were one complete syllable, had only one pronunciation, and did not correspond to any other word on the list in terms of spelling or pronunciation (Loveman et al. 2002). This test was scored based on percent correct.

Visual Recall: In this test, a computer-generated list of 10 random letters was presented, one at a time, on a computer screen at a frequency of one letter/second. At the end of the list, participants were asked to type in as many of the letters as they could recall. This test was scored automatically and expressed as percent correct (Young and Benton 2013).

Reaction Test: Participants were instructed to hold their hand out at their side and to cup their hand to form a horizontal U with their thumb and pointer finger. A meter stick was placed just above the U and was released. The goal of the participants was to squeeze the meter stick between the thumb and pointer finger as soon as the meter stick was released. Distance along the meter stick was recorded with highest numbers representing slowest reaction times. The average traveled distance (cm) of the meter stick from three trials was recorded.

Blood Glucose: Left middle fingers were pricked with SurgiLance® lancets. Blood glucose levels were obtained using the ReliOn® blood glucose monitoring equipment.

Stroop Test: Participants performed a computerized 96-word Stroop test in which they were instructed to report the physical color of the word, rather than the word itself. Accuracy and time to complete the test was recorded.

Electroencephalograph (EEG) and Heart Rate: Participants were connected to an ADI Data Acquisition System with an EEG recording lead and a heart rate monitor. The EEG electrodes were placed on the posterior aspect of the head along the midsagittal line at the level of the ears, and two more electrodes were placed on the forehead one inch on either side of the midsagittal line. The plethysmograph was placed on the participant's right middle finger. Resting heart rate was recorded during a four-minute resting baseline. Alpha and beta brain waves were recorded during the four-minute baseline and during the Stroop test.

STATISTICAL ANALYSIS

All data were expressed as percent of baseline. The three time periods were analyzed by ANOVA and if a significant F value was obtained post hoc comparisons of selected means were assessed by t-test. All statistical tests were performed at a 95 percent confidence interval.

Results

The effects of caffeine over time are grouped as functions of mood, physiological effects or measured cognitive effects. Baseline values did not differ significantly between groups for any parameter tested, and all analyses (except where indicated) were expressed as percent change from baseline values at 2.5 and five hours. Significance differences among treatment groups at each time period were examined by an ANOVA, followed by post hoc testing of a t-test, $p<0.05$.

Mood

Relaxed/Stress: Scores ranged from 21 to 99 with an average of 71.8 +/- 2.55, indicating placement on the relaxed end of the scale. There were no significant differences in reported stress levels between groups at any time.

Happy/Sad: Scores ranged from 32-100 with a baseline average of 76.5 +/- 2.06 indicating placement on the happy end of the scale. There was no change in happiness in the placebo group for any time period. Although all caffeine groups rated a happier mood at 2.5 hours post-consumption, and decreased to baseline levels by the five-hour time; this rise was only significant in the CP group (123.76 +/- 7.86 percent of baseline) and this group reported being significantly happier than the SDS and placebo groups at 2.5 hours (Figure 1).

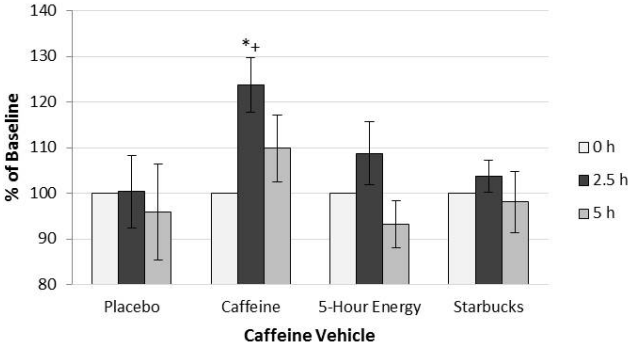

FIGURE 1. Happiness ratings of participants indicating a signficant increase at 2.5 hours in the CP group, where * indicates significant difference from baseline; + indicates significant difference from other groups.

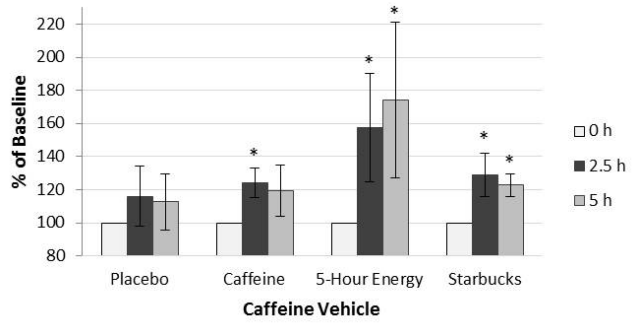

FIGURE 2. Participant perception of focus indicated significant (*) increases for all caffeine groups after 2.5 hours which was sustained for 5HE and SDS for five hours.

Focus/Distracted: Scores ranged from 12-92 with the average of all participants being 58.8 +/- 2.59 indicating placement slightly toward the focused side of the scale. The focus/distracted mood response pattern was similar to that of happy/sad mood level changes, with no change in the placebo group over time, while all caffeine groups reported a significantly greater percent change in focus after 2.5 hours compared to baseline (CP: 124.16 +/- 8.94 percent, 5HE: 157.38 +/- 32.87 percent, and SDS: 128.96 +/- 13.02 percent). Only the SDS and 5HE sustained significant focus from baseline at five hours post-consumption (Figure 2).

Awake/Tired: Scores ranged from seven to 96 with a mean of 40.85 +/- 3.39 for all groups placing them toward the tired end of the scale. All groups, including the placebo group, became significantly more alert at 2.5 hours post consumption, which is to be expected over the course of the morning. To eliminate normal increase in wakefulness, all treatment groups were expressed as a percent of placebo at the equivalent time. The 5HE group reported significantly higher perception of alertness at 2.5 hours, which was sustained for five hours (469.51 +/- 160.80 percent and 395.55 +/- 123.55 percent of baseline respectively). The CP group also reported a significant sustained increase in alertness (2.5 hour: 153.14 +/- 23.03 percent, five hours: 142.63 +/- 24.49 percent of baseline) (Figure 3).

Memory

Auditory Recall: Auditory recall scores ranged from six to 65 percent recall with an average of 39.9 +/- 1.35 percent. To eliminate habituation effects,

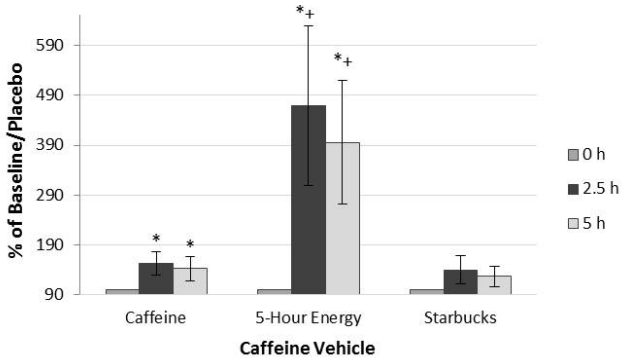

FIGURE 3. Relative self-reported alertness expressed as percent of placebo. * indicates significant difference from baseline within groups and + indicates significant difference from other groups.

each treatment group was expressed as a percent of placebo at the equivalent time point. At 2.5 hours, no treatment groups showed a significant increase in recall scores, but the SDS showed a significant decrease (91.26 +/- 5.13 percent of baseline) that was sustained at five hours, dropping to 88.24 percent +/- 5.84 percent of baseline. However, the 5HE group showed a significant increase in auditory recall score at five hours (122.90 percent +/- 9.32 percent of baseline), which differed significantly from the other treatment groups (Figure 4).

Visual Recall: Visual recall scores at baseline ranged from 50-100 percent with an average of 72.7 percent +/- 1.63 percent. Placebo group scores did not change significantly. To eliminate habituation effects, each treatment group was expressed as a percent of placebo at the equivalent time point. No significant difference was found with memory between groups at any time.

Reaction Time

Baseline distance traveled before the participant caught the ruler during the reaction time ranged from 12.7 cm to 36.83 cm, averaging 25.65 +/- 0.40 cm. The placebo group had no significant change in reaction time. At 2.5 hours, participants in all caffeine groups significantly improved their reaction times (CP: 81.91 +/- 3.74 percent, 5HE: 76.68 +/- 6.12 percent and SDS: 75.02 percent +/- 3.55 percent of baseline) which were sustained at five hours post-consumption (Figure 5).

Blood Glucose

Although students were asked to fast for 12 hours prior to testing, there were considerable variations in baseline blood glucose levels, ranging from 70 mg/dL to 130 mg/dL with an average of 96.5 mg/dL +/- 1.60 mg/dL. Participants in the placebo group experienced a significant rise (106.5 +/- 2.82 percent of baseline) in blood glucose 2.5 hours after consuming the placebo and the bagel, which then dropped significantly below baseline at five hours to 92.07 +/- 1.88 percent of baseline. The 5HE group exhibited a significant increase (111.21 +/- 3.86 percent) from baseline at 2.5 hours, which dropped significantly at five hours, but this change was still significantly higher than the placebo and SDS groups. A significant increase at 2.5 hours was not observed in the CP and SDS groups, but the SDS groups' blood glucose level significantly decreased at five hours post-consumption (Figure 6).

Resting Heart Rate

Initial resting heart rates ranged from 59-115 bpm with a mean of 73.7 +/- 1.61 bpm. To eliminate habituation effects, each treatment group was expressed as a percent of placebo at the equivalent time point. At five hours post consumption, the CP and 5HE groups' heart rates rose significantly to 109.9 +/- 2.49 percent and 109.06 +/- 3.97 percent of baseline, respectively, and were significantly higher than the SDS group at five hours (Figure 7).

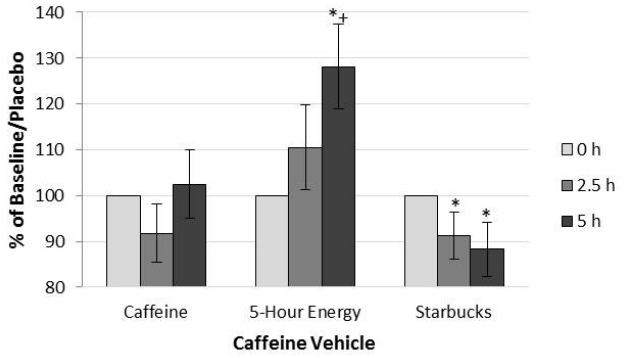

FIGURE 4. Auditory recall expressed as a percent of placebo. * indicated significant difference within group over time. + indicates significant difference from other groups.

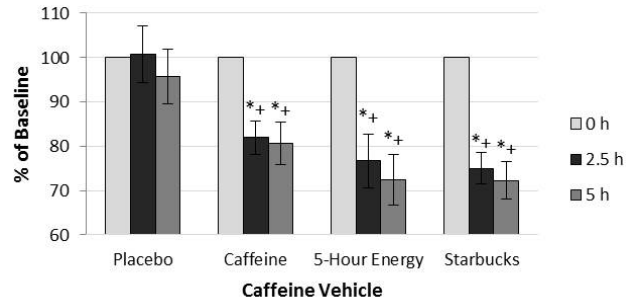

FIGURE 5. Reaction time as percent of initial distance traveled before being grabbed where * indicates significance within group and + indicates that groups are significantly different from placebo.

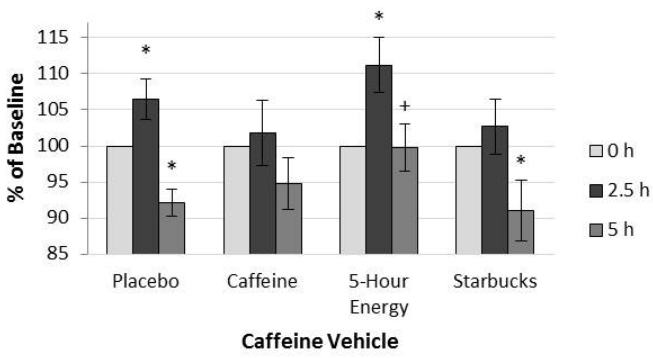

FIGURE 6. Circulating blood glucose levels expressed as percent of baseline, where * indicates significance within group and + indicates significant difference from placebo and SDS groups.

Stroop Test

Time: Time to complete the Stroop test ranged from 69.3 to 159.7 seconds with an average of 98.9 +/- 2.63 seconds. A learning curve was observed such that completion time significantly decreased for all groups. However, all the caffeine groups significantly decreased the time to complete the Stroop test when compared to the placebo at all testing time points (Figure 8).

Accuracy: Stroop accuracy at baseline ranged from 86-96 percent with an average of 93.75 percent. A significant learning curve was seen in all groups, so caffeine treatments were calculated as percent of placebo to determine the portion of the effect attributable to caffeine. The CP and 5HE groups were significantly more accurate at 2.5 hours (101.27 +/- 0.70 percent and 101.10 +/- 0.58 percent of baseline respectively); but this effect was not sustained (Figure 9).

Brain Waves

Adhesion issues were experienced which impacted electrode connections, therefore; subject's data (15 of 60) who were two standard deviations from the mean due to adhesion problems were excluded from analysis. As in the alertness survey, all treatment groups for all the waves were expressed as a percent of placebo at the equivalent time to eliminate normal increase in wakefulness.

Resting Alpha Waves: Initial resting alpha waves recorded during testing at baseline ranged from 5.9 – 51.54 mV with an average of 16.97 +/- 1.40 mV. The 5HE group had significantly decreased alpha waves at all testing times compared to baseline and to the other treatment groups dropping to 61.58 +/- 12.37 perecent and 75.84 +/- 11.62 percent of baseline at 2.5 and five hours respectively (Figure 10).

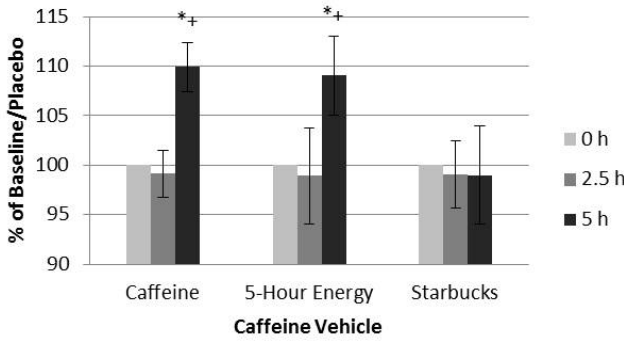

FIGURE 7. Resting heart rate as a percent of initial rate where * indicates significance with group and + indicates significant difference from SDS group.

Stroop Alpha Waves: Initial alpha waves recorded during Stroop testing at baseline ranged from 7.84 – 167.87 mV with an average of 39.69 +/- 5.51 mV. The 5HE group had significantly decreased alpha waves at all times compared to the other treatment groups. At 2.5 hours, it significantly decreased from baseline to 38.86 +/- 10.61 percent, but this effect was not sustained (Figure 10).

Resting Beta Waves: Initial resting beta waves ranged from 5.72 to 24.01 mV with an average of 14.05 mV, with no significant difference in measured resting beta waves among any groups at any time (Figure 10).

Stroop Beta Waves: Initial beta waves recorded during Stroop testing ranged from 11.70 to 80.58 mV with an average of 27.84 +/- 2.92 mV. At 2.5 hours, the 5HE group had significantly decreased beta waves (63.78 +/- 16.67 percent of baseline), but this effect was not sustained. The CP and SDS group did not show any significant changes in Stroop beta waves (Figure 10).

Overall, the greatest increases were seen in the 5HE group and that the majority of these were sustained more effectively in 5HE than in other caffeine delivery methods (Table 2).

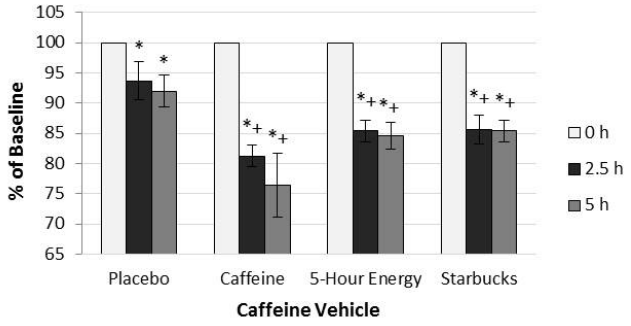

FIGURE 8. Time to complete Stroop test expressed as a percent of baseline where * indicates significance within group and + indicates significant difference from placebo.

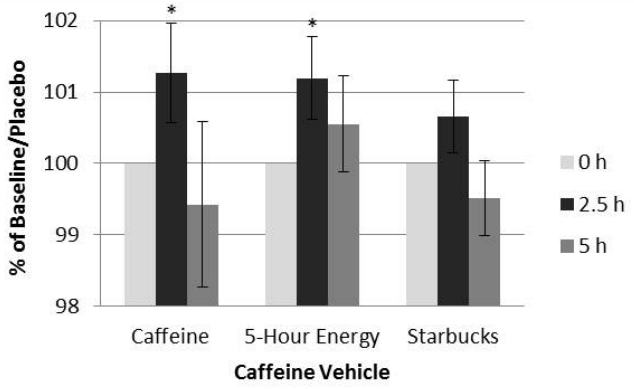

FIGURE 9. Stroop accuracy was expressed as percent of placebo with * indicating significant difference within the group.

DISCUSSION

SDS had little long-term caffeine effect and improved only focus in terms of mood. However, SDS consumption improved both physical reaction time and the time to complete the Stroop test, which has been previously shown (Patat et al. 2000; Haskell et al. 2005; Adan and Serra-Grabulosa 2010; Dixit et al. 2012; Souissi et al. 2012, 2013; Pilli et al. 2013; Santos et al. 2014). Those who consumed SDS had the poorest performance on the Stroop test (time and accuracy) compared to the other groups and auditory recall scores were significantly worse at each time period. SDS did not impair glucose homeostasis as seen in previous studies (Young and Benton 2013; Beaudoin et al. 2013; Cooper et al. 2014), nor did it alter heart rate, (which is consistent with Giles et al. (2012) and Bichler et al. (2006) or brain wave activity.

The biggest difference between SDS and other caffeine types is the additional glucose content of the SDS. Synergistic activity of the glucose/caffeine combination has demonstrated a degree of synergy in the short-term by increasing attention and energy and decreasing reaction time (Adan and Serra-Grabulosa, 2010; Serra-Grabulosa et al. 2010; Scholey and Kennedy, 2004), but an antagonist effect has been observed longer term (Young and Benton, 2013). Benton et al. (2003) suggested that consuming foods with a high glycemic index resulted in subjects being less energetic as the morning went on and subjects who consumed foods with a low glycemic index resulted in better memory. Therefore, the high glycemic index of SDS could be causing a "sugar crash" that is impairing extended performance. Furthermore, glucose may have inhibited caffeine absorption as has been suggested by Childs, (2014). Overall, the SDS group did not perform significantly better than the other caffeine groups and had the lowest number of sustained effects.

The CP group (caffeine alone) exhibited significant short term improvements in focus, alertness, and happiness at 2.5 hours post-treatment, as has been previously seen (Arciero et al., 2009; Rogers et al., 2008; Haskell et al., 2005; Smit et al., 2004; Loke, 1988) but only alertness was sustained. Furthermore, the CP group had the slowest reaction times of all the caffeine groups. A pilot study, by this research group, using the same dosing of caffeine showed a significant improvement in time to complete the Stroop test at 30 minutes post consumption and this study found

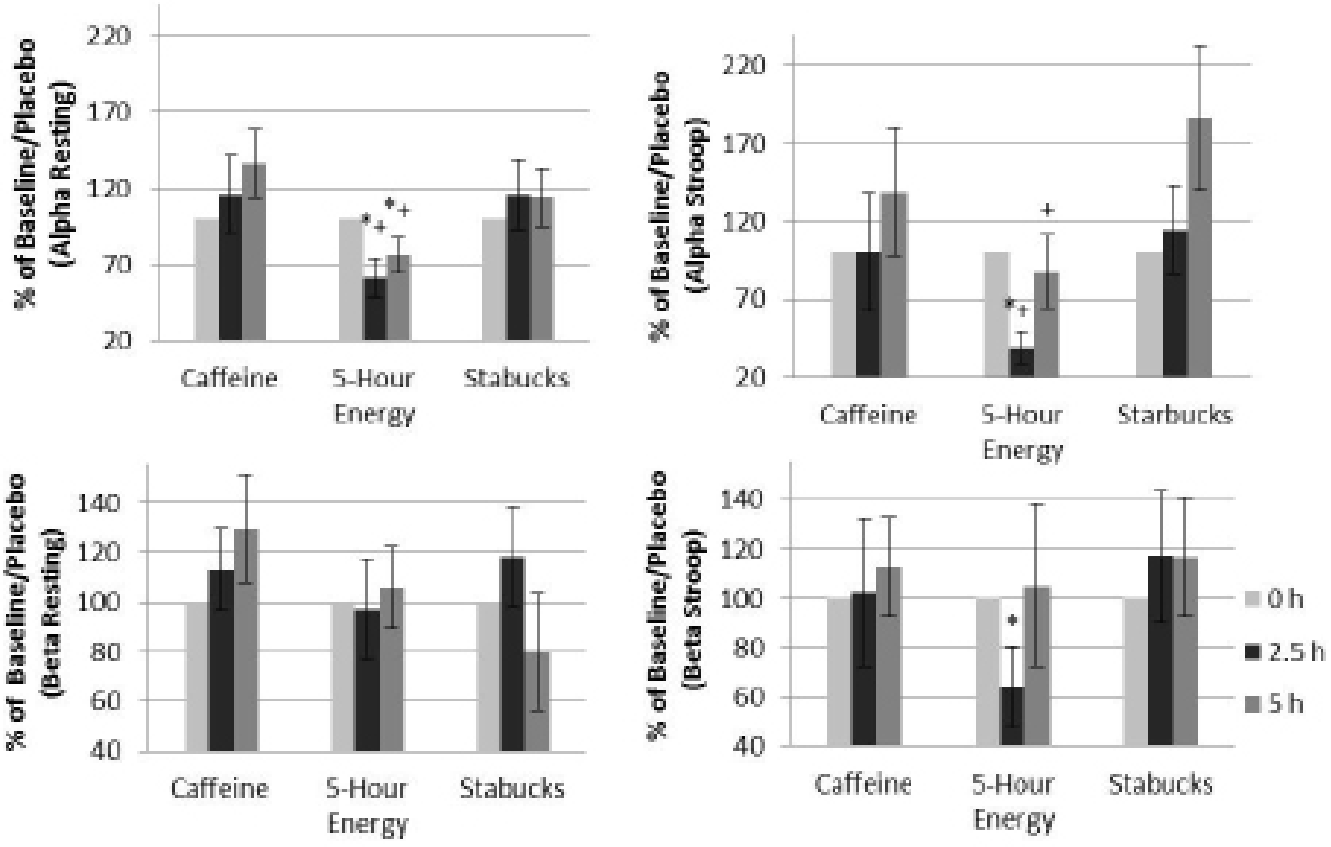

FIGURE 10. Alpha (above) and beta (below) brain waves at rest (left) and during Stroop testing (right) were expressed as percent of placebo with * indicating significant difference within the group and + indicating difference from groups.

that the increase in Stroop accuracy and speed at 2.5 hours was not sustained. CP like SDS, did not alter glucose homeostasis or brain wave activity nor did it improve memory as has been reported by Nehlig, (2010), Smith, (2002), and Loke, (1988). At 2.5 hours, no change in heart rate was observed, which is consistent with Giles et al. (2012) and; Bichler et al. (2006), therefore, the elevated heart rate observed at five hours was likely not a caffeine effect. Subjects in the CP group had the highest happiness scores and the fastest time to complete the Stroop test, but it does not appear that CP had a sustained impact on mood or mental acuity.

5HE improved focus and alertness but not happiness through the five hour duration of the study. Giles et al. (2012) and Peacock et al. (2013) found that caffeine

TABLE 2
Timing Sequence of Caffeine Effects for Three Vehicles of Caffeine Delivery

	Caffeine		5-Hour Energy®		Starbucks DoubleShot®	
	2.5 Hrs	5 Hrs	2.5 Hrs	5 Hrs	2.5 Hrs	5 Hrs
Mood						
Happy/Sad	+*	0	0	0	0	0
Focus/Distracted	+	0	+	+	+	+
Awake/Tired	+	+	+*	+*	0	0
Physiological Effects						
Reaction Time	+*	+*	+*	+*	+*	+*
Blood Glucose	0	0	+	+*	0	--
Resting Heart Rate	0	+*	0	+*	0	0
Cognitive Effects						
Auditory Memory	0	0	+	+*	--	--
Stroop Time	--*	--*	--*	--*	--*	--*
Stroop Accuracy	+	0	+	0	0	0
Neurological Effects						
Resting Alpha Wave	0	0	--*	--*	0	0
Stroop Alpha Waves	0	0	--*	--*	0	0
Stroop Beta Waves	0	0	--	0	0	0

+ indicates significant increase from baseline; -- indicates significant decrease from baseline; 0 indicates no significant change; and * indicates significant difference from other groups.

could be inhibited by taurine, an ingredient in 5HE. 5HE was similar to CP in that it decreased reaction time and time to complete the Stroop test and exhibited a similar trend in heart rate with no short-term effect and a later increase in HR. Like the CP group, 5HE also improved Stroop accuracy at 2.5 hours, but was not sustained. The inability to sustain the Stroop accuracy is most likely contributed to the effects of caffeine beginning to wear off. The 5HE group exhibited elevated blood glucose levels throughout the study indicating an impairment of glucose metabolism; and was the only group exhibiting long term improvement in auditory recall. A 2013 5HE study (Wesnes et al., 2013) yielded similar results where memory peaked at two hours and declined after three hours, thus the 2.5 hour measure of this study may represent waning effects of caffeine.

In both the Wesnes et al. (2013) study and this study, memory is re-elevated at five hours consistent with improvements in short-term memory (90-minutes post-consumption) after caffeine consumption as reported by Young and Benton (2013). Therefore, any memory improvement directly related to caffeine may have declined before the first 2.5 hour test point of this longer term study, implying that another ingredient in 5HE is causing improvements in memory at five hours. 5HE was the only treatment to significantly decrease both alpha and beta brain waves at all time periods. This is consistent with caffeine impact on alpha waves (Foxe et al. 2012; Barry et al. 2008, 2009, 2010; Keane et al. 2007; Siepmann et al. 2002; Gilbert 2000; Dimpfel et al., 1993). The brain wave data almost parallels the 5HE's alertness scores, which strengthens the claim that 5HE significantly increases alertness over all other treatment groups either by sustaining the caffeine effect or by some other factor not determined in this study. Studies on beta waves are inconsistent, with some researchers reporting an increase in beta waves (Sigmon et al. 2009; Keane and James 2008; Patat et al. 2000), while others found a decrease (Barry et al. 2009; Siepmann et al. 2002; Gilbert 2000; Dimpfel, et al. 1993).

CONCLUSION

Caffeine taken alone causes improvements in mood and cognitive performance up to 2.5 hours and the effects can be felt as long as five hours after consumption, but when caffeine is taken with glucose the results are hampered. However, when taken in 5HE performance and mood are improved and sustained, indicating the5HE was the best energy enhancer tested in this study. Something in the 5HE not only sustains the caffeine effect, but also enhances its effect. The 5HE performed significantly better than the other caffeine groups on self-reported alertness, decreases in alpha waves, and on the memory test. The 5HE also had more sustained effects through five hours including decreases in alpha waves, memory, reaction time, Stroop time, and self-reported levels of focus and alertness. It was also the only caffeine group to show caffeine's effect on alpha and beta waves, memory improvements, and blood glucose.

The component of the 5HE increasing caffeine efficacy was not explored in this present experiment partially because the ingredients are proprietary. B Complex vitamins have been linked to long term effects, but neither the acute effects nor interaction with caffeine have been studied. Citicoline has been reported to reduce cognitive decline in elderly, but it too has not been studied acutely or in combination with caffeine (Childs, 2014). Taurine alone has shown beneficial effects over a placebo, but it has attenuated caffeine induced effects on mood (Peacock et al. 2013; Giles et al. 2012). Other amino acids in 5HE have been linked to cognitive improvements when taken alone. For example Tyrosine counteracts adverse effects from stress and fatigue by increasing attention, mood and memory (Mahoney et al. 2007; O'Brien et al. 2007; Magill et al. 2003), but an interaction with caffeine has not been investigated. L-theanine, a common amino acid found in tea, has a synergist effect with caffeine on alertness and simple reaction time (Haskell et al. 2008).

Future studies should examine the interaction of each component with caffeine and determine the ingredients and concentrations of 5HE's "energy blend." Until such an experiment is conducted it will remain unknown what ingredient in 5HE is responsible for the increased duration and efficacy. Additionally, future studies should address several of the limitations presented here including: improved EEG electrode adhesion, a more accurate way to test reaction time, larger sample sizes, and greater restrictions on the participants.

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LITERATURE CITED

Adan A. Serra-Grabulosa J. 201). Effects of caffeine and glucose, alone and combined, on cognitive performance. Hum Psychopharmacol Clin. 25(4): 310-317. doi:10.1002/hup.1115.

Alford C, Cox H, Wescott R. 2001. The effects of red bull energy drink on human performance and mood. Amino Acids. 21(2): 139-150.

Arciero PJ, Ormsbee MJ. 2009. Relationship of blood pressure, behavioral mood state, and physical activity following caffeine ingestion in younger and older women. App Physiol Nutr Metab. 34(4): 754-762.

Babb MM, Wald LL, Cohen MM, Villafuerte AA, Gruber AA, Yurgelun-Todd AA, Renshaw FF. 2002. Chronic citicoline increases phosphodiesters in the brains of healthy older subjects: an in vivo phosphorus magnetic resonance spectroscopy study. Psychopharmacol. 161(3): 248.

Barry RJ, Clarke AR, Johnstone SJ, Brown CR, Bruggemann JM, van Rijbroek I. 2009. Caffeine effects on resting-state arousal in children. Int J Psychophysiol. 73(3): 355-361.

Barry RJ, Clarke AR, Johnstone SJ, Rushby JA. 2008. Timing of caffeine's impact on autonomic and central nervous system measures: Clarification of arousal effects. Biol Psychol. 77(3): 304-316.

Barry RJ, Clarke AR, Johnstone SJ. 2011. Caffeine and opening the eyes have additive effects on resting arousal measures. Clin Neurophysiol. 122(10): 2010-2015

Beaudoin M, Allen B, Mazzetti G, Sullivan P, Graham T. 2013. Caffeine ingestion impairs insulin sensitivity in a dose-dependent manner in both men and women. App Physiol Nutr Metab. 38(2): 140-147.

Bello D. 2008. Energy drinks: Sales soar, concerns rise. Family Safety Health. 67(2): 24-25.

Benton D., Ruffin M., Lassel T., Nabb S., Messaoudi M., Vinoy S., Desor D., Lang V. 2003). The delivery rate of dietary carbohydrates affects cognitive performance in both rats and humans. Psychopharmacology. 166(1): 86.

Bichler A, Swenson A, Harris MA. 2006. A combination of caffeine and taurine has no effect on short term memory but induces changes in heart rate and mean arterial blood pressure. Amino Acids. 31(4): 471-476.

Bottoms L, Greenhalgh A, Gregory K. 2013. The effect of caffeine ingestion on skill maintenance and fatigue in epee fencers. J Sports Sci. 31(10): 1091-1099.

Bryan J, Calvaresi E, Hughes D. 2002. Short-term Folate, Vitamin B-12 or Vitamin B-6 supplementation slightly affects memory performance but not mood in women of various ages. J Nutr. 132(6): 1345-1356.

Childs, E. 2014. Influence of energy drink ingredients on mood and cognitive performance. Nutr Rev. 72(1): 48-59.

Cooper R, Naclerio F, Allgrove J, Larumbe-Zabala E. 2014. Effects of a carbohydrate and caffeine gel on intermittent sprint performance in recreationally trained males. Eur J Sport Sci. 14(4): 353-361.

Dimpfel W, Schober F, Spüler M. 1993. The influence of caffeine on human EEG under resting conditions and during mental loads. Clin Investigator. 71(3): 197-207.

Dixit A, Goyal A, Thawani R, Vaney N. 2012. Effect of caffeine on information processing: evidence from Stroop task. Indian J Psychol Med. 34(3): 218-222.

Foxe JJ, Morie KP, Laud PJ, Rowson MJ, de Bruin EA, Kelly SP. 2012. Assessing the effects of caffeine and theanine on the maintenance of vigilance during a sustained attention task. Neuropharmacology. 62(7): 2319-2326.

García-Cobos, R., Frank-García, A., Gutiérrez-Fernández, M., Díez-Tejedor, E. 2010. Citicoline, use in cognitive decline: Vascular and degenerative. J Neurol. Sci. 299(1/2): 188-192. doi:10.1016/j.jns.2010.08.027.

Gilbert DG. 2000. Effects of nicotine and caffeine, separately and in combination, on EEG topography, mood, heart rate, cortisol, and vigilance. Psychophysiology. 37(5): 583-595.

Giles G, Mahoney CR, Brunyé TT, Gardony AL, Taylor HA, Kanarek RB. 2012. Differential cognitive effects of energy drink ingredients: caffeine, taurine, and glucose. Pharmacol Biochem Be.. 102(1): 569–577.

Glade M. 2010. Caffeine-Not just a stimulant. Nutr J. 26(10): 932-938.

Haskell CF, Kennedy DO, Wesnes KA, Scholey AB. 2005. Cognitive and mood improvements of caffeine in habitual consumers and habitual non-consumers of caffeine. Psychopharmacol. 179(4): 813-825.

Haskell CF, Robertson B, Jones E, Forster J, Jones R, Wilde A, Kennedy DO. 2010. Effects of a multi-vitamin/mineral supplement on cognitive function and fatigue during extended multi-tasking. Hum Psychopharmacol. 25(6): 448-461.

Haskell C F, Kennedy D O, Milne A L, Wesnes K A, Scholey AB. 2008. The effects of l-theanine, caffeine and their combination on cognition and mood. Biol Psychol. 77(2): 113-122. doi:10.1016/j.biopsycho.2007.09.008.

Hätönen KA, Virtamo J, Eriksson JG, Sinkko HK, Erlund I, Jousilahti P, Valsta, L. M. 2012. Coffee does not modify postprandial glycaemic and insulinaemic responses induced by carbohydrates. Eur J Nutr. 51(7): 801-806.

Keane MA, James JE, Hogan MJ. 2007. Effects of Dietary Caffeine on Topographic EEG after Controlling for Withdrawal and Withdrawal Reversal. Neuropsychobiology. 56(4): 197-207.

Keane MA, James JE. 2008. Effects of dietary caffeine on EEG, performance and mood when rested and sleep restricted. Hum Psychopharmacol. 23(8): 669-680.

Kennedy DO, Haskell CF. 2011. Vitamins and cognition, what is the evidence Drugs. 71(15): 1857-1971.

Kennedy D O, Haskell C F, Robertson B B, Reay J J, Brewster-Maund C C, Luedemann, J J, & Scholey B. 2008. Improved cognitive performance and mental fatigue following a multi-vitamin and mineral supplement with added guaraná (Paullinia cupana). Appetite. 50(2/3): 506-513. doi:10.1016/j.appet.2007.10.007.

Loke, WH. 1988. Effects of caffeine on mood and memory. Physiol & Behav 44(3): 367–372.

Loveman E, van Hooff JC, Gale A. 2002. A systematic investigation of same and cross modality priming using written and spoken responses. Memory. 10(4): 267-276.

Magill RA, Waters WF, Bray GA, Volaufova J, Smith SR, Lieberman HR, Ryan DH. 2003. Effects of tyrosine, phentermine, caffeine d-amphetamine, and placebo on cognitive and motor performance deficits during sleep deprivation. Nutr Neurosci. 6(4): 237.

Mahoney CR, Castellani J, Kramer F, Young A, Lieberman HR. 2007. Tyrosine supplementation mitigates working memory decrements during cold exposure. Physiol & Behav. 92(4): 575-582.

Malinauskas BM, Aeby VG, Overton RF, Carpenter-Aeby T, Barber-Heidal K. 2007. A survey of energy drink consumption patterns among college students. Nutr J. 6(1) 635-641.

McDaniel M, Maier S, Einstein G. 2003. Brain-specific nutrients: a memory cure? Nutr. 19(11/12): 957-975

McIlvain GE; Noland MP; Bickel R. 2011. Caffeine consumption patterns and beliefs of college freshmen. Am. J Health Edu. 42(4), 235-244.

Mclellan, T M, Lieberman H R. 2012. Do energy drinks contain active components other than caffeine? Nutrition Reviews. 70(12), 730-744.

National Library of Medicine. Caffeine in the diet: MedlinePlus Medical Encyclopedia. U.S National Library of Medicine. U.S., n.d. Web. 27 Nov. 2014. <http://www.nlm.nih.gov/medlineplus/ency/article/002445.htm>.

Nawrot P, Jordan S, Eastwood J, Rotstein J, Hugenholtz A, Feeley M. 2003. Effects of caffeine on human health. Food Addit. Contam. 20(1): 1-30.

Nehlig, A. 2010. Is Caffeine a Cognitive Enhancer? J Alzheimers Dis. 20(1):85-94.

O'Brien C, Mahoney C, Tharion WJ, Sils IV, Castellani JW. 2007. Dietary tyrosine benefits cognitive and psychomotor performance during body cooling. Physiol & Behav. 90(2/3): 301-307

Patat A, Rosenzweig P, Enslen M, Trocherie S, Miget N, Bozon MC, Allain H, Gandon JM. 2000. Effects of a new slow release formulation of caffeine on EEG, psychomotor and cognitive functions in sleep-deprived subjects. Hum Psychopharmacol. 15(3): 153-170.

Peacock A, Martin F, Carr A. 2013. Energy drink ingredients. Contribution of caffeine and taurine to performance outcomes. Appetite. 64(1):1-4.

Pilli R, MUR N, Rani Pingali U, Shobha J, Reddy A. 2013. A computerized Stroop test for the evaluation of psychotropic drugs in healthy participants. Indian J Of Psychol Med. 35(2): 180-189.

Poltev V, Rodríguez E, Grokhlina T, Deriabina A, Gonzalez E. 2010. Computational study of the molecular mechanisms of caffeine action: Caffeine complexes with adenosine receptors. Int J Quantum Chem. 110(3): 681-688.

Rogers PJ, Smith JE, Heatherley SV, Pleydell-Pearce CW. 2008. Time for tea: mood, blood pressure and cognitive performance effects of caffeine and theanine administered alone and together. Psychopharmacol. 195(4): 569-577.

Santos V, Felippe L, Almeida J, Bertuzzi R, Kiss M, Lima-Silva A. 2014. Caffeine reduces reaction time and improves performance in simulated-contest of taekwondo. Nutr J. 6(2): 637-649.

Scholey AB, Kennedy DO. 2004. Cognitive and physiological effects of an "energy drink": an evaluation of the whole drink and of glucose, caffeine and herbal flavouring fractions. Psychopharmacology. 176(3/4): 320-330.

Serra-Grabulosa JM, Adan A, Falcón C, Bargalló N. 2010. Glucose and caffeine effects on sustained attention: an exploratory fMRI study. Hum Psychopharmacol. 25(7/8): 543-552.

Siepmann M, Kirch W. 2002. Effects of caffeine on topographic quantitative EEG. Neuropsychobiology. 45(3): 161-166.

Sigmon S, Herning R, Better W, Cadet J, Griffiths R. 2009. Caffeine withdrawal, acute effects, tolerance, and absence of net beneficial effects of chronic administration: cerebral blood flow velocity, quantitative EEG, and subjective effects. Psychopharmacol. 204(4): 573-585.

Smit HJ, Cotton JR, Hughes SC, Rogers PJ. 2004. Mood and cognitive performance effects of "energy" drink constituents: caffeine, glucose and carbonation. Nutr Neurosci. 7(3): 127-139.

Smith A. 2002. Effects of caffeine on human behavior. Food Chem Toxicol. 40(1): 1243–1255

Souissi M, Abedelmalek S, Chtourou H, Atheymen R, Hakim A, Sahnoun Z. 2012. Effects of morning caffeine' ingestion on mood states, simple reaction time, and short-term maximal performance on elite judoists. Asian J Sports Med. 3(3): 161-167.

Souissi M, Abedelmalek S, Chtourou H, Boussita A, Hakim A, Sahnoun Z. 2013. Effects of time-of-day and caffeine ingestion on mood states, simple reaction time, and short-term maximal performance in elite judoists. Biol Rhythm Research. 44(6): 897-907.

Sünram-Lea S, Owen-Lynch J, Robinson S, Jones E, Hu H. 2012. The effect of energy drinks on cortisol levels, cognition and mood during a fire-fighting exercise. Psychopharmacol. 219(1): 83-97.

Terry WS, Phifer B. 1986. Caffeine and memory performance on the AVLT. J Clin Psychol. 42(6): 860-863.

Wesnes KA, Barrett ML, Udani JK. 2013. An evaluation of the cognitive and mood effects of an energy shot over a 6h period in volunteers. A randomized, double-blind, placebo controlled, cross-over study. Appetite. 67(1): 105-113.

Young HH, Benton D. 2013. Caffeine can decrease subjective energy depending on the vehicle with which it is consumed and when it is measured. Psychopharmacology. 228(2): 243-254.