Effects of Time-Release Caffeine Containing Supplement on Metabolic Rate, Glycerol Concentration and Performance

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Abstract
This study compared caffeine pharmacokinetics, glycerol concentrations, metabolic rate, and performance measures following ingestion of a time-release caffeine containing supplement (TR-CAF) versus a regular caffeine capsule (CAF) and a placebo (PL). Following a double-blind, placebo-controlled, randomized, cross-over design, ten males (25.9 ± 3.2 y) who regularly consume caffeine ingested capsules containing either TR-CAF, CAF, or PL. Blood draws and performance measures occurred at every hour over an 8-hour period. Plasma caffeine concentrations were significantly greater (p < 0.05) in CAF compared to TR-CAF during hours 2-5 and significantly greater (p = 0.042) in TR-CAF compared to CAF at hour 8. There were no significant differences between trials in glycerol concentrations (p = 0.86) or metabolic measures (p = 0.17-0.91). Physical reaction time was significantly improved for CAF at hour 5 (p=0.01) compared to PL. Average upper body reaction time was significantly improved for CAF and TR-CAF during hours 1-4 (p = 0.04 and p = 0.01, respectively) and over the 8-hour period (p = 0.04 and p = 0.001, respectively) compared to PL. Average upper body reaction time was also significantly improved for TR-CAF compared to PL during hours 5-8 (p = 0.004). TR-CAF and CAF showed distinct pharmacokinetics yielding modest effects on reaction time, yet did not alter glycerol concentration, metabolic measures, or other performance measures.

Key words: Caffeine bioavailability, energy, multiple object tracking, pharmacokinetics, reaction time, sustained release caffeine.

Introduction

Caffeine is the most widely used pharmacologically active compound, and it has been estimated that 80 - 90% of users report habitual consumption, with a daily average intake of approximately 200 - 250 mg (Barone and Roberts, 1996; Juliano and Griffiths, 2004). Caffeine has been shown to enhance cognitive function and feelings of mental alertness, mood, and arousal (Goldstein et al., 2010; Nehlig, 2010). In addition, caffeine ingestion has been demonstrated to maintain or enhance vigilance and choice reaction time (Lieberman et al., 2002; Mclellan et al., 2005), and is commonly used to alleviate the effects of sleep deprivation and fatigue (Beaumont et al., 2001; De Valck and Cluydts, 2001; Penetar et al., 1993).

Previous investigations have determined that caffeine is completely absorbed by the gastrointestinal tract within 1 hour, with peak plasma concentrations occurring between 15 and 120 minutes following ingestion (Magkos and Kavouras, 2005). Plasma caffeine concentrations have also been shown to rise in a dose-dependent manner and exhibit first order, linear kinetics resulting in a half-life of approximately 5 hours (Bonati et al., 1982; Kamimori et al., 2002). However, the half-life of caffeine has also been reported to range between 2.5 and 10 hours by other investigators (Magkos and Kavouras, 2005). Additionally, the nature of formulation can also directly influence the rate and extent of absorption following oral administration as caffeine has shown to have a greater rate of absorption from a capsule than from dietary sources such as coffee (Fredholm et al., 1999), cola, or chocolate (Mumford et al., 1996).

The efficacy of time-release caffeine capsules appears to be no different than regular caffeine capsules. Investigations have demonstrated that time-release caffeine can enhance alertness and reaction performance for up to 13 hours following ingestion (Lagarde et al., 2000), and improve vigilance and cognitive function during sleep deprivation as compared to a placebo (Beaumont et al., 2001; 2004; 2005; De Valck and Cluydts, 2001; De Valck et al., 2003; Doireau et al., 1997; Lagarde et al., 2000; Patat et al., 2000; Sicard et al., 1996). However, these previous studies have not compared the efficacy of time-release caffeine directly to regular caffeine capsule ingestion, nor have they examined performance changes relative to differences in the pharmacokinetics of caffeine uptake into the plasma. We hypothesized that a time-release caffeine containing supplement would alter the pharmacokinetics of caffeine, creating a sustained plateau of caffeine concentration in the plasma following consumption. In addition, the sustained effect of time-release caffeine may prolong the lipolytic, metabolic, and performance effects generally associated with caffeine ingestion.

Thus the primary objective of this study was to compare the pharmacokinetics of a multi-ingredient time-release caffeine containing supplement (TR-CAF) versus an equivalent dose of a regular caffeine capsule and a placebo. In addition, glycerol concentration, metabolic rate, reactive and cognitive performance, and subjective

measures of mood, energy, focus, and alertness were assessed during an 8-hour period following ingestion.

Methods

Participants

Ten males (25.9 ± 3.2 y; 1.81 ± 0.08 m; 92.9 ± 9.9 kg; 13.3 ± 3.6 % body fat) volunteered to participate in this acute randomized, double-blind, placebo-controlled study. Following an explanation of all procedures, risks, and benefits, each participant gave his informed consent prior to participation in this study. The Institutional Review Board of the University of Central Florida approved the research protocol. For inclusion in the study, participants had to be regular caffeine consumers (>200 mg per day) to increase homogeneity of the sample. Participants were excluded if they had any history of cardiovascular disease, metabolic, renal, hepatic, or musculoskeletal disorders or were taking any other medication as determined by a questionnaire.

Protocol

Participants reported to the Human Performance Laboratory (HPL) for one familiarization session prior to experimental trials. During the familiarization session, participants were informed of all procedures and familiarized with all performance measures to reduce the possibility of a learning effect. Participants performed three trials with seven days between each trial. During each trial, participants consumed either a multi-ingredient supplement containing time-release caffeine (TR-CAF); a regular caffeine supplement (CAF); or a placebo (PL).
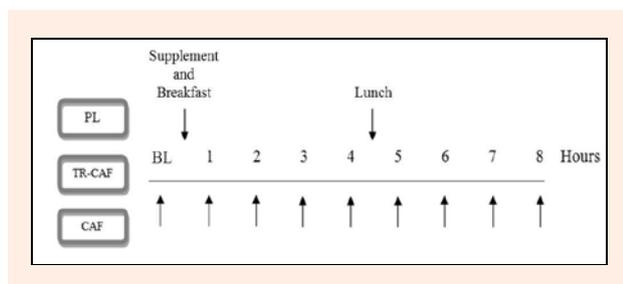

Figure 1. Study Protocol. The experimental trials were randomized into one of three trials: placebo (PL); time-release caffeine supplement (TR-CAF); and a regular caffeine supplement (CAF). Participants reported following an 8-hour fast and were instructed not to exercise 24 hours prior. Assessments took place at baseline (prior to supplement ingestion) and at each hour following ingestion of the supplement for a total of 8 hours. Assessments consisted of blood measures, metabolic measures, cardiovascular measures, subjective measures, and performance measures. Between assessments, participants sat comfortably in a quiet room without distraction wearing noise cancelling headphones and were provided a standardized breakfast and lunch.

Participants reported to the HPL at 0800 hour following an 8-hour fast and were instructed not to exercise 24 hours prior to each trial. Assessments took place at baseline (prior to supplement ingestion) and at each hour following ingestion of the supplement for a total of 8 hours. Assessments consisted of blood measures, metabolic measures, cardiovascular measures, subjective measures, and performance measures. Between assessments, participants sat comfortably in a quiet room without distraction wearing noise cancelling headphones (Bose, QuietComfort® 15, Framingham, MA). Participants were provided a standardized breakfast (310 kcal; 45 g carbohydrate, 17 g protein, 6 g fat) and lunch (290 kcal; 38 g carbohydrate, 19 g protein, 7 g fat) and were permitted to drink water ad libitum. The study protocol is depicted in Figure 1.

Supplement

The caffeine containing supplements (TR-CAF and CAF) and PL were ingested in tablet form, and two tablets were consumed during each trial. Tablets for all trials were similar in appearance and taste. The TR-CAF supplement contained 194 mg time-release caffeine, 5.2 mg vitamin B1, 25 mg vitamin B6, 200 µg folate, 3 µg vitamin B12, 150 mg magnesium, 971 mg L-tyrosine, 250 mg glucuronolactone, 75 mg theobromine, 75 mg rhodiola rosea extract, 25 mg Korean ginseng powder, and 10 mg octacosanol. To compare the supplement with an equivalent amount of regular caffeine, CAF contained 194 mg regular caffeine and rice powder, while the PL contained rice powder only. CAF served as a typical caffeine ingestion ordinarily used by habitual caffeine users.

Blood measurements

During each experimental trial, all blood samples were obtained using a 20-gauge Teflon cannula placed in a superficial forearm vein using a three-way stopcock with a male luer lock adapter. The cannula was maintained patent using an isotonic saline solution (Becton Dickinson, Franklin Lakes, NJ). The first blood draw occurred at baseline (BL) prior to supplementation and breakfast. Following ingestion of the supplement and breakfast, blood draws occurred at every hour over the 8 hour period (9 total blood draws). Each participant's blood samples were obtained at the same time of day during each session.

All blood samples were collected into two Vacutainer® tubes, one containing SST® Gel and Clot Activator and the second containing sodium heparin. The sodium heparin tube was kept chilled prior to each blood draw. The blood in the first tube was allowed to clot at room temperature for 2 hours and subsequently centrifuged at 3,000×g for 15 min along with the remaining whole blood from the second tube. The resulting plasma and serum was placed into separate 1.8-mL microcentrifuge tubes and frozen at −80°C for later analysis.

Biochemical analysis

Plasma caffeine concentrations were quantified using high performance liquid chromatography (HPLC). Chromatographic conditions were based upon a modified version of Agilent Technologies application brief (Agilent Technologies, Santa Clara, CA). Chromatography was performed on an Agilent Infinity 1260 HPLC (Agilent Technologies, Santa Clara, CA) consisting of a degasser, binary pump, auto-sampler, column thermostat, and photodiode array detector. A Zorbax Eclipse Plus C18 (4.6 x 150mm, 5-µm) column and Zorbax analytical guard column (4.6 x 12.5 mm, 5-µm) were used for separation. Data were collected using OpenLAB chromatography data system,

ChemStation edition.

All reagents were of HPLC grade. Caffeine, beta-hydroxyethyl-theophylline, sodium phosphate monobasic and sodium phosphate dibasic were purchased from Sigma-Aldrich (St. Louis, MO) to create the stock solution. Acetonitrile was purchased from Fisher Scientific (Pittsburgh, PA). HPLC grade water was prepared by reverse-osmosis and purified using a Milli-Q Direct 8 water purification system (EMD Millipore, Billerica, MA).

A 40 $\mu g \cdot mL^{-1}$ stock solution of caffeine, theobromine and beta-hydroxyethyl-theophylline was prepared in water and sonicated. Twelve calibration standards were prepared from the stock solution in the range of 0.039 – 40 $\mu g \cdot mL^{-1}$ by serial dilution of 1 mL of the stock solution. Beta-hydroxyethyl-theophylline (internal standard; IS) working solution was prepared in water (10 $\mu g \cdot mL^{-1}$).

An internal plasma sample was collected to serve as control and analyzed every 50 samples. Calibration standards, samples, and controls were prepared in the same fashion for linearity. Sixty microliters of the calibration standards or 50 μL of sample or quality control sample was added to 1.5 mL microcentrifuge tubes. Ten microliters of IS was subsequently added to the samples and controls, followed by 140 μL of chilled acetonitrile for deproteinization. Standards, samples, and controls were then vortexed vigorously for 30 seconds and placed in a refrigerator (4 ℃) for two hours followed by centrifugation at 14000g for 15 minutes in a microcentrifuge to allow the protein to form a pellet. The supernatant (150 μL) was collected and subsequently transferred to a 0.45 μm polytetrafluoroethylene syringeless filter vial (GE Healthcare Mini-Uniprep™, Piscataway, NJ). A concentration of 300 μL of sodium phosphate buffer (mobile phase) was then added to the vial. The solution was filtered and injected into the HPLC using an auto-sampler.

The mobile phase consisted of 25 mM sodium phosphate (pH 7.0 ± 0.05 at 40 ℃) and acetonitrile at a volume to volume ratio of 90:10. Buffer pH was achieved by mixing 4.77 g sodium phosphate monobasic anhydrous, and 7.79 g sodium phosphate dibasic anhydrous in 3.785 L of water at 40 ℃. Buffer composition was calculated using Buffer Maker computer software (Marki, Poland) and verified using an Oakton pH 11 portable meter (Oakton Instruments, Vernon Hills, IL). Analysis was carried out under isocratic conditions via binary mixing of aqueous and organic phases at a flow rate of 1.5 $mL \cdot min^{-1}$ under a system pressure of approximately 90 bars. Chromatograms were recorded at 275 nm with a run time of 6 minutes. Duplication of retention times for a known standard was used to verify column equilibrium prior to analysis.

Glycerol concentrations were determined using an automated analyzer (Analox GM7 enzymatic metabolite analyzer, Analox instruments USA, Lunenburg, MA). To eliminate inter-assay variance, all samples for a particular assay were thawed once and analyzed in the same assay run by a single technician. All samples were run in duplicate with a mean intra-assay variance of 11.6%.

Metabolic measures

Resting metabolic rate (RMR) was measured in the laboratory using a ventilation hood (ParvoMedics TrueOne Metabolic System OUSW 4.3.4). RMR was determined by measuring O_2 consumption and CO_2 production. Participants were instructed to rest in the supine position in a recliner, to minimize movements, and to remain awake during the measurement period. Participants remained under the ventilation hood for 15 minutes at each assessment time point. The first 5 minutes of the reading were excluded from analysis. RMR was calculated as the average of the readings during the remaining 10 minutes. Measurements of VO_2 ($ml \cdot min^{-1}$), VCO_2 ($ml \cdot min^{-1}$), respiratory quotient (RQ), and resting energy expenditure (REE) ($kcal \cdot day^{-1}$) were recorded. Machine calibration was performed prior to each testing session.

Cardiovascular measures

Average heart rate and blood pressure were measured at each assessment time point using a wireless heart rate monitor (Polar® RS800CX, Kempele, Finland) and a digital blood pressure monitor (Omron Healthcare, Inc, HEM-712C, Vernon Hills, Illinois). Average heart rate was measured during the 15 minutes under the ventilation hood. Blood pressure was measured directly following the removal of the ventilation hood.

Subjective measures

Participants were instructed to assess their subjective feelings of energy, alertness, and focus using a 15-cm visual analog scale (VAS). The scale was anchored by the words "Lowest" and "Highest" to represent extreme ratings where the greater measured value represents the greater feeling. Questions were structured as "My level of energy is", "My level of alertness is", and "My level of focus is". The validity and reliability of VAS in assessing subjective feelings have been previously established (Lee et al., 1991). In addition, participants were also instructed to complete a profile of mood states (POMS) questionnaire (Mcnair et al., 1971). The POMS consisted of 58 words or phrases in a Likert format questionnaire that provided measures of specific mood states including vigor, tension, depression, anger, fatigue, and confusion. A total mood score was calculated by subtracting vigor from the sum of the 5 other negative measures and adding 100 to avoid a negative result. Measures of consistency ranging between 0.85 and 0.95 and test-retest reliability estimates ranging between 0.65 and 0.74 have been previously reported for the POMS instrument (Mcnair et al., 1971).

Performance measures

Upper body reaction measurements: Measurement of upper body reaction time was performed on the Dynavision D2 Visuomotor Training Device (D2; Dynavision International LLC, West Chester, OH). The D2 is a light-training reaction device developed to train sensory motor integration through the visual system (Wells et al., 2014). It consists of a board (4 foot x 4 foot) that can be raised or lowered relative to the height of the participant. It contains 64 target buttons (lights) arranged into five concentric circles surrounding a center screen that can be illuminated to serve as a stimulus for the par-

ticipant. Participants were required to assume a comfortable athletic stance and stand at a distance from the board where they can easily reach all of the lights. The board height was kept consistent for all testing trials and was adjusted per participant so the center screen was located just below eye level. A total of three different reaction tests were conducted.

The first assessment measured the participant's ability to react to a stimulus (light) as it changed position on the board. An initial stimulus (light) was present on the D2 in a random location. The stimulus (light) remained lit until it was touched by the participant. A stimulus (light) then appeared at another random location. The participant was instructed to successfully identify and touch as many stimuli (lights) as possible within 60 seconds. The number of successful "hits" was recorded for each trial. The ICC of this test has shown to be 0.75 in our laboratory (Wells et al., 2014).

The second assessment was similar to the previous measure in that participants were also required to react to a visual stimulus (light) as it changed position on the board. However, during this trial the stimulus (light) remained lit for 1 second before it changed to another random location and the participant had to verbally recite a five digit number that was presented on the center screen of the D2 every 5 seconds. The appearance of the digits placed a cognitive demand on the information processing resources of the participant. The participant was instructed to successfully identify and touch each stimulus before it changed position and score as many touches as possible within 60 seconds. The number of successful "hits" was recorded for each trial. The ICC of this test has shown to be 0.73 in our laboratory (Wells et al., 2014).

The third assessment measured the participant's visual, motor, and physical reaction times to a visual stimulus with the dominant hand. The test was initiated when the participant placed and held his hand on an illuminated "home" button. At this point, a stimulus (light) was presented randomly in one of five locations, parallel to the home button. Visual reaction time was measured as the amount of time it takes to identify the stimulus (light) and initiate a reaction by taking their hand off the home button. Motor response time was measured as the amount of time it takes to physically touch the stimulus (light) with their hand following the initial visual reaction and was measured as the amount of time between the hand leaving the home button and touching the stimulus (light). Physical reaction time was measured as the total elapsed time from the introduction of the target stimulus (light) to the physical completion of the task (returning to the home button after touching the stimulus).All measures were recorded to the 1/100's of a second. Participants performed this assessment ten times. The average time for all ten assessments was recorded. In our laboratory, the ICC of this test has shown to be 0.84 (visual) and 0.63 (motor) (Wells et al., 2014).

Lower body reaction measurements: Lower body reaction time was measured using a 20-second reaction test on the Quick Board™ (The Quick Board, LLC, Memphis, TN) reaction timer. Participants stood on a board of five circles in a 2 x 1 x 2 pattern. Participants

straddled the middle circle and reacted to a visual stimulus located on a display box that depicts one of five potential lights that corresponded with the circles on the board. Upon illumination of a light, the participant attempted to move the dominant foot to the circle that corresponds to the visual stimulus. Upon a successful "hit" with the foot, the next stimulus appeared. The total number of successful attempts during the 20-second test and the average time between the activation of the light and the response to the corresponding circle was recorded. Test-retest reliability for the Quick Board™ in our laboratory has consistently shown r>0.90.

Multiple object tracking and cognitive assessments: Multiple object tracking was assessed using a Cave Automatic Virtual Environment (CAVE) system. The CAVE consists of a 7 ft × 7 ft × 7 ft room that includes a canvas projection screen on the front wall which served as the surface for image projection. During each session, the participant wore three dimensional glasses. A three-dimensional image of 8 tennis balls was projected onto the front screen. The participant tracked 4 of the 8 balls that moved in three-dimensions. At the beginning of each trial, the 8 balls appeared frozen on the screen for 2 seconds while half of them turned grey indicating the balls the participant was to track. After the 2 seconds, the balls all became the same color again and began to move in three dimensions. At the conclusion of the trial (8 seconds), the balls froze and a number appeared on each ball. The participant called out the numbers of the four balls he was supposed to be tracking. Velocity of movement began at a slow tracking speed and increased or decreased depending on whether the participant correctly identified the 4 correct balls. Each participant performed 20 trials per session. The velocity of movement that was most successful was recorded. The ICC of this test has shown to be 0.77 in our laboratory (Fragala et al., 2014).

A modified version of the original Serial Sevens Test was also utilized to analyze cognitive function. This test consisted of a two-minute timed oral test in which participants were required to subtract the number 7 from a random computer generated four digit number, in order to measure how quickly and accurately they could compute a simple mathematical problem. The computer generated numbers were written onto standard note cards. Participants were given a randomized stack of note cards and asked to complete as many calculations as possible in a two minute period. The participant and scorer sat opposite each other during testing. The answers to the calculations were written on the back of the note cards in pencil for the scorer to see. Participants were not able to see the correct answer. Once the participant released the note card, their answer was considered unchangeable. The number of correct answers and the average time per correct answer was recorded.

Statistical analysis

Changes from baseline measures were analyzed using repeated measures analysis of covariance (ANCOVA) with the BL measure serving as the covariate. In the event of a significant F ratio, LSD post-hoc tests were used for pairwise comparisons using adjusted means. For bio
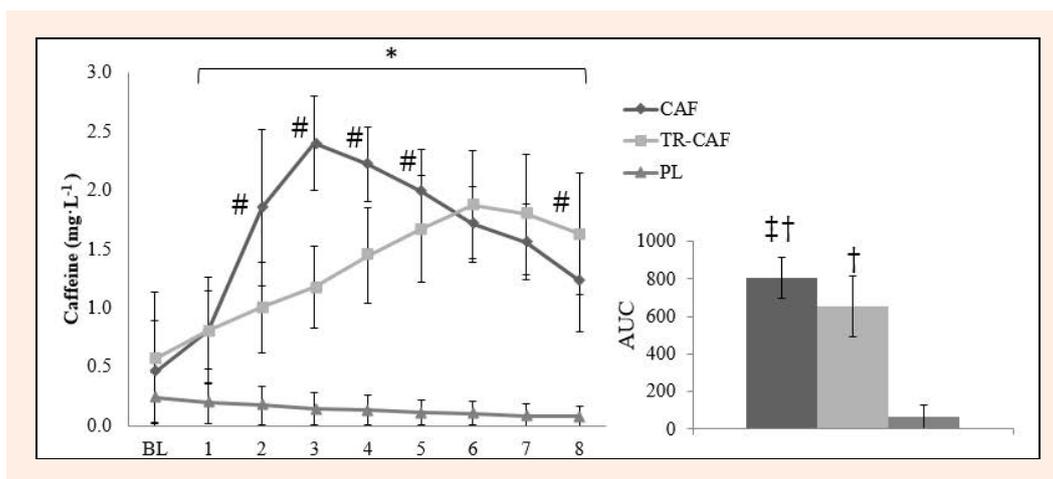
Figure 2. Plasma caffeine concentration was measured at baseline (prior to supplement ingestion) and at each hour following ingestion of the supplement for a total of 8 hours. PL = Placebo; TR-CAF = Time-release caffeine supplement; CAF = Regular caffeine supplement; AUC = Area under curve * Plasma caffeine concentration for CAF and TR-CAF was significantly greater than PL ($p < 0.001$). # Plasma caffeine concentration was significantly different between CAF and TR-CAF ($p < 0.05$). † CAF and TR-CAF showed significantly greater plasma caffeine concentration AUC than PL over the 8 hours ($p < 0.0001$). ‡ CAF showed significantly greater plasma caffeine concentration AUC than TR-CAF over the 8 hours ($p = 0.001$).

chemical and metabolic measures, area under the curve (AUC) was calculated using a standard trapezoidal technique. AUC analysis was analyzed using ANCOVA for all 8 hours, hours 1 - 4, and hours 5 - 8. Performance measures were also averaged for hours 1 - 8, hours 1 - 4, and hours 5 - 8 and analyzed using ANCOVA. Results were considered significant at an alpha level of $p \leq 0.05$. All data are reported as mean ± SD.

Results

Biochemical analysis
Changes in plasma caffeine concentrations can be observed in Figure 2. Significant differences between trials were observed for plasma caffeine concentrations over the 8 hours. Plasma caffeine concentrations for CAF and TR-CAF were significantly greater than PL ($p < 0.001$) at hours 1 - 8. In addition, plasma caffeine concentrations for CAF were significantly greater ($p < 0.05$) than TR-CAF between hours 2 - 5, while plasma caffeine concen-

trations were significantly greater ($p = 0.04$) in TR-CAF compared to CAF at hour 8. AUC analysis revealed that plasma caffeine concentrations for CAF and TR-CAF were significantly greater than PL at hours 1 - 4 ($p < 0.0001$), hours 5 - 8 ($p < 0.0001$), and over the 8 hour study duration ($p < 0.0001$). Additionally, AUC for CAF was significantly greater than TR-CAF for hours 1 - 4 ($p < 0.0001$) and over the 8 hour study duration ($p < 0.001$).

Changes in plasma glycerol concentrations can be observed in Figure 3. There were no significant differences between trials in plasma glycerol concentrations ($p=0.86$). Additionally, there were no significant differences between trials for AUC for glycerol concentrations for hours 1 - 4 ($p = 0.27$), hours 5 - 8 ($p = 0.14$), and over the 8 hour study duration ($p=0.11$). When data were collapsed among the trials, a significant decrease from BL was observed at hour 2 ($p = 0.04$), while significant increases above BL were noted at hours 5 and 6 ($p=0.04$ and $p = 0.03$, respectively) for plasma glycerol concentrations.
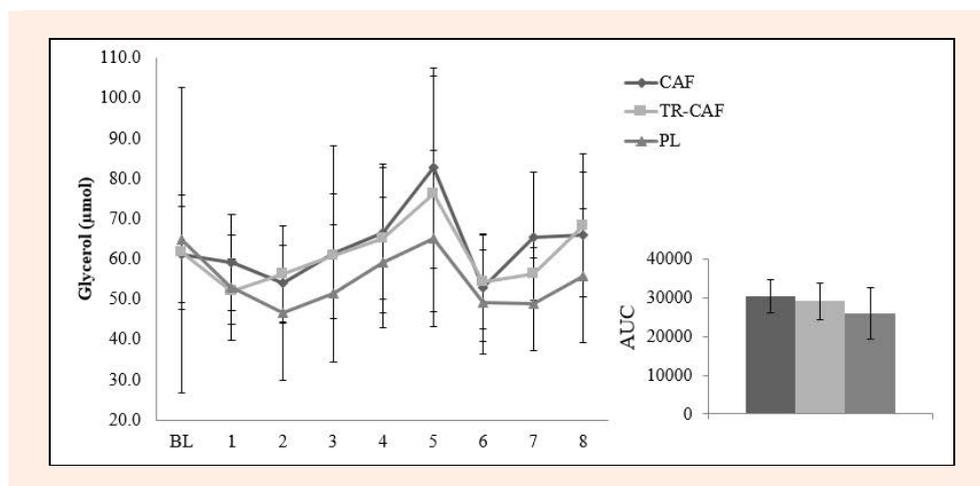

Figure 3. Glycerol concentration was measured at baseline (prior to supplement ingestion) and at each hour following ingestion of the supplement for a total of 8 hours. PL = Placebo; TR-CAF = Time-release caffeine supplement; CAF = Regular caffeine supplement; AUC = Area under curve

Table 1. Metabolic and cardiovascular measures were measured at baseline (prior to supplement ingestion) and at each hour following ingestion of the supplement for a total of 8 hours. Data are means (±SD).

	VO$_2$ (ml•min^{-1})								
	BL	1	2	3	4	5	6	7	8
CAF	297 (30)	331 (32)	312 (47)	301 (28)	311 (38)	329 (24)	313 (31)	312 (31)	324 (34)
TR-CAF	288 (27)	331 (34)	312 (36)	298 (26)	301 (23)	337 (35)	313 (24)	310 (27)	318 (29)
PL	293 (25)	337 (34)	299 (24)	288 (22)	285 (22)	324 (34)	304 (21)	306 (23)	309 (30)
		*	*			*	*	*	*

	VCO$_2$ (ml•min^{-1})								
	BL	1	2	3	4	5	6	7	8
CAF	245 (25)	294 (38)	284 (39)	257 (24)	259 (27)	274 (17)	272 (26)	270 (28)	278 (25)
TR-CAF	238 (17)	289 (32)	273 (38)	256 (23)	251 (22)	283 (25)	278 (31)	272 (23)	273 (30)
PL	245 (27)	300 (38)	266 (18)	248 (21)	238 (19)	278 (35)	272 (15)	275 (24)	272 (27)
		*	*	*		*	*	*	*

	Respiratory Quotient								
	BL	1	2	3	4	5	6	7	8
CAF	.83 (.05)	.88 (.05)	.90 (.06)	.85 (.06)	.84 (.06)	.84 (.06)	.87 (.04)	.87 (.04)	.86 (.02)
TR-CAF	.83 (.05)	.87 (.05)	.87 (.07)	.86 (.06)	.84 (.06)	.84 (.04)	.89 (.08)	.88 (.05)	.86 (.05)
PL	.84 (.05)	.89 (.05)	.89 (.05)	.86 (.05)	.84 (.05)	.86 (.07)	.90 (.04)	.90 (.05)	.88 (.05)
		*	*	*			*	*	*

	Resting Energy Expenditure (kcal•day^{-1})								
	BL	1	2	3	4	5	6	7	8
CAF	2058 (198)	2326 (233)	2231 (271)	2099 (189)	2158 (248)	2281 (145)	2185 (213)	2177 (214)	2257 (231)
TR-CAF	1995 (170)	2313 (237)	2184 (253)	2080 (170)	2085 (152)	2338 (232)	2197 (170)	2174 (182)	2216 (206)
PL	2034 (177)	2368 (248)	2099 (154)	2008 (149)	1977 (148)	2261 (235)	2138 (138)	2153 (163)	2167 (203)
		*	*			*	*	*	*

	Heart Rate (beats•min^{-1})								
	BL	1	2	3	4	5	6	7	8
CAF	57 (11)	60 (11)	62 (11)	57 (10)	60 (10)	64 (13)	59 (10)	58 (10)	61 (11)
TR-CAF	56 (10)	59 (9)	59 (10)	57 (10)	59 (9)	61 (11)	60 (9)	59 (10)	62 (11)
PL	58 (13)	62 (12)	59 (10)	56 (9)	57 (10)	62 (12)	61 (8)	59 (10)	59 (10)
		*	*			*	*	*	*

	Systolic Blood Pressure (mmHg)								
	BL	1	2	3	4	5	6	7	8
CAF	120 (12)	125 (12)	124 (11)	124 (11)	123 (9)	124 (13)	119 (13)	121 (13)	124 (15)
TR-CAF	120 (13)	123 (15)	121 (11)	120 (13)	120 (8)	121 (12)	124 (11)	122 (12)	123 (11)
PL	122 (14)	122 (11)	120 (11)	120 (15)	121 (10)	123 (9)	121 (12)	123 (11)	122 (11)

	Diastolic Blood Pressure (mmHg)								
	BL	1	2	3	4	5	6	7	8
CAF	64 (8)	67 (6)	69 (5)	70 (9)	68 (9)	63 (9)	65 (9)	68 (7)	70 (5)
TR-CAF	66 (8)	66 (10)	67 (7)	67 (9)	66 (6)	65 (8)	68 (6)	67 (9)	69 (10)
PL	67 (8)	65 (11)	67 (6)	67 (6)	67 (6)	66 (7)	67 (7)	66 (11)	68 (6)

PL=Placebo; TR-CAF= Time-release caffeine supplement; CAF=Regular caffeine supplement. * Significant increase (p<0.05) above BL, when data were collapsed across trials.

Metabolic measures

Metabolic measures can be observed in Table 1. There were no significant differences between trials for changes in VO$_2$ (p = 0.17), VCO$_2$ (p = 0.66), RQ (p = 0.91), or REE (p = 0.18) over the 8 hour study duration. In addition, AUC analysis revealed no significant differences between trials for any of the metabolic measure between hours 1 - 4 (p = 0.34-0.73), hours 5 - 8 (p = 0.26-0.67), and over the 8 hours (p = 0.32-0.37).

When data were collapsed across trials, a significant increase above BL for VO$_2$ was seen at hours 1, 2, and 5 - 8 (p = 0.0001-0.004), and a significant increase above BL for VCO$_2$ was seen between hours 1 - 3 and 5 - 8 (p = 0.0001-0.03). Collapsed data also showed a significant increase above BL for RQ between hours 1 - 3 and 6 - 8 (p = 0.0001-0.01), and a significant increase above BL for REE at hours 1, 2, and 5 - 8 (p < 0.0001).

Cardiovascular measures

There were no significant differences between trials for changes in average heart rate (p = 0.36), systolic blood pressure (p = 0.78), or diastolic blood pressure (p = 0.74). When data were collapsed across trials, a significant increase above BL was noted for heart rate at hours 1, 2, and 5 - 8 (p = 0.0001-0.02) (see Table 1). Systolic and diastolic blood pressure values did not significantly differ from BL (120.4 ± 12.5 and 65.9 ± 7.7 mmHg, respectively) over the 8 hour study duration.

Subjective measures

There were no significant differences between trials for changes in feelings of energy (p = 0.92), alertness (p = 0.85), or focus (p = 0.69). There were also no significant differences between trials for average energy, alertness, or focus for hours 1 - 4 (p=0.23-0.45), hours 5 - 8 (p=0.29-0.50), and over the 8 hour study duration (p = 0.24-0.43). Additionally, there were no significant differences between trials for changes in mood states including tension (p = 0.52), depression (p = 0.34), anger (p = 0.44), vigor (p = 0.96), fatigue (p = 0.72), confusion (p = 0.35), or total mood score (p = 0.72).

Table 2. **Average upper body reaction times were measured using the Dynavision D2 Visuomotor Training Device at baseline (prior to supplement ingestion) and at each hour following ingestion of the supplement for a total of 8 hours. Visual reaction time was measured as the amount of time it takes to identify the stimulus (light) and initiate a reaction by taking their hand off the home button. Motor response time was measured as the amount of time it takes to physically touch the stimulus (light) with their hand following the initial visual reaction and was measured as the amount of time between the hand leaving the home button and touching the stimulus (light). Physical reaction time is a measurement of the total elapsed time from the introduction of the target stimulus (light) to the physical completion of the task (returning to the home button after touching the stimulus). Data are means (±SD).**

	Visual Reaction Time (s)								
	BL	1	2	3	4	5	6	7	8
CAF	.35 (.05)	.34 (.04)	.34 (.04)	.34 (.04)	.33 (.04)	.33 (.04)	.33 (.04)	.34 (.05)	.33 (.04)
TR-CAF	.36 (.06)	.35 (.05)	.34 (.05)	.33 (.04)	.34 (.04)	.33 (.04)	.33 (.04)	.33 (.05)	.34 (.06)
PL	.35 (.05)	.34 (.05)	.33 (.04)	.35 (.05)	.34 (.05)	.35 (.05)	.34 (.04)	.33 (.05)	.33 (.04)
			*		*	*	*	*	*
	Motor Reaction Time (s)								
	BL	1	2	3	4	5	6	7	8
CAF	.20 (.03)	.19 (.03)	.20 (.06)	.21 (.04)	.20 (.04)	.18 (.04)	.20 (.06)	.20 (.06)	.20 (.05)
TR-CAF	.21 (.04)	.20 (.04)	.22 (.04)	.22 (.06)	.18 (.04)	.20 (.03)	.23 (.06)	.23 (.07)	.21 (.04)
PL	.22 (.06)	.23 (.06)	.25 (.07)	.24 (.08)	.23 (.06)	.24 (.07)	.20 (.04)	.22 (.04)	.21 (.07)
	Physical Reaction Time (s)								
	BL	1	2	3	4	5	6	7	8
CAF	.55 (.05)	.54 (.04)	.54 (.08)	.54 (.06)	.54 (.06)	.51 (.06)[#]	.54 (.09)	.54 (.07)	.53 (.07)
TR-CAF	.56 (.08)	.55 (.06)	.54 (.08)	.55 (.07)	.52 (.06)	.53 (.05)	.55 (.08)	.56 (.10)	.55 (.08)
PL	.57 (.08)	.57 (.07)	.58 (.08)	.59 (.09)	.57 (.08)	.59 (.09)	.54 (.07)	.55 (.07)	.54 (.08)

PL=Placebo; TR-CAF= Time-release caffeine supplement; CAF=Regular caffeine supplement. # Significantly decreased (p<0.05) compared to PL. * Significant decrease (p<0.05) from BL, when data were collapsed across trials.
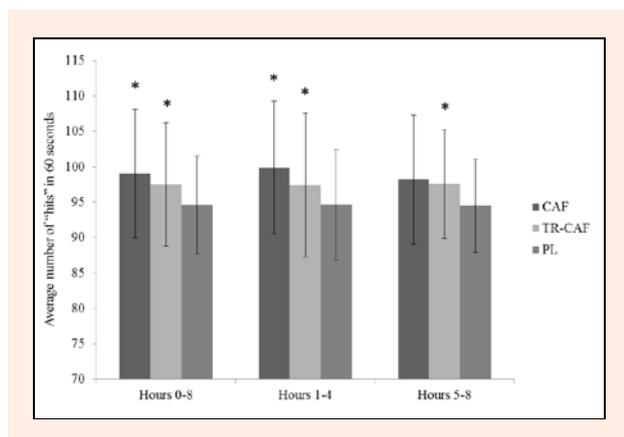

Figure 4. Upper body reaction time was measured as the number of successful "hits" in 60 seconds using the Dynavision D2 Visuomotor Training Device (D2; Dynavision International LLC, West Chester, OH). Assessment took place at baseline (prior to supplement ingestion) and at each hour following ingestion of the supplement for a total of 8 hours. Values were averaged for all 8 hours, hours 1-4, and hours 5-8. Values are means ± SD. PL=Placebo; TR-CAF= Time-release caffeine supplement; CAF=Regular caffeine supplement. * Significant difference compared to PL (p<0.05)

Performance measures

Changes in visual, motor and physical reaction times can be seen in Table 2. A significant difference was observed between trials for mean physical reaction. Subsequent post-hoc analysis revealed that mean physical reaction time was significantly faster for CAF at hour 5 (p = 0.01) compared to PL. No other significant changes were noted in visual, motor, or physical reaction times between the groups. When data were collapsed across trials, a significant decrease from BL was noted for visual reaction time at hours 2 and 4 - 8 (p = 0.001-0.02).

The average number of "hits" in 60 seconds was significantly greater for CAF and TR-CAF compared to PL for hours 1 - 4 (p = 0.04 and p = 0.01, respectively) and over the 8 hour study period (p = 0.04 and p = 0.001, respectively) (see Figure 4). The average number of "hits" in 60 seconds was also significantly greater for TR-CAF compared to PL for hours 5 - 8 (p = 0.004). No significant differences between trials were observed for upper body reaction with a cognitive demand (p = 0.45) and values did not significantly differ from BL (85.0 ± 15.5 "hits") over the 8 hour study duration. No significant differences between trials were observed for lower body reaction (p = 0.43) and values did not significantly differ from BL (29.8 ± 2.9 "hits") over the 8 hour study duration. Additionally, no significant differences between trials were observed for multiple object tracking (p = 0.23) and values did not significantly differ from BL (1.9 ± 0.5 AU) over the 8 hour study duration. No significant differences between trials were observed for the cognitive function test (p = 0.56). However, when data were collapsed across trials, values were significantly greater (p < 0.0001) than BL (39.4 ± 12.9 correct answers) for hours 1 - 8.

Discussion

The results of this study indicate that a multi-ingredient supplement containing 194 mg of time-release caffeine resulted in a slower rate of caffeine absorption compared to a similar dose of regular caffeine. Both caffeine supplements modestly improved upper body reaction time, yet did not alter glycerol concentrations, metabolic or cardiovascular measures, or subjective measures of mood in habitual caffeine consumers. Additionally, other performance measures, including lower body reaction time, multiple object tracking, and cognitive function were not affected by either caffeine supplement.

This appears to be the first study to compare plasma caffeine pharmacokinetics of a time-release caffeine

containing supplement directly to a regular caffeine capsule ingestion. Although plasma caffeine concentrations for both caffeine supplements were significantly greater than PL over the 8 hour study duration, CAF rapidly reached peak plasma caffeine concentration (2.40 ± 0.40 $mg \cdot L^{-1}$) at 3 hours following ingestion, while TR-CAF reached peak plasma caffeine concentration (1.88 ± 0.46 $mg \cdot L^{-1}$) at 6 hours following ingestion. AUC analysis revealed that plasma caffeine concentrations were significantly greater following CAF ingestion during hours 1 - 4 compared to TR-CAF, however, there was no difference in the plasma caffeine AUC during hours 5 - 8. Additionally, TR-CAF had a significantly greater plasma caffeine concentration compared to CAF at hour 8. Previous research has measured salivary caffeine concentrations following the ingestion of 600 mg of time-release caffeine capsules in habitual caffeine consumers yielding a peak caffeine concentration of 7.66 ± 0.19 $mg \cdot L^{-1}$ (Sicard et al., 1996). The saliva-to-plasma concentration ratio has been reported to be approximately 0.74 ± 0.08 (Newton et al., 1981). Following ingestion of 200 mg of regular caffeine, peak plasma caffeine concentrations of 4.13 ± 1.92 $mg \cdot L^{-1}$ have been reported at ~2 hours following ingestion (Kamimori et al., 2002). Kamimori et al. (2002) also showed that the peak plasma caffeine concentration and time-to-peak increased in a linear dose dependent manner when comparing 50, 100, and 200 mg caffeine capsules. Therefore, a greater dose of TR-CAF may be necessary to increase caffeine bioavailability.

Heart rate and blood pressure were not significantly altered by CAF or TR-CAF in the current study. This is consistent with other studies examining caffeine ingestion and cardiovascular changes. Previous investigations have not reported any cardiovascular abnormalities for 13 hours following ingestion of up to 600 mg of time-release caffeine (Lagarde et al., 2000; Patat et al., 2000). Additionally, heart rate has shown to be unaffected by regular caffeine doses of up to 400 mg per serving (Astrup et al., 1990). Astrup et al. (1990) also showed significant elevations in systolic and diastolic blood pressure following administration of 400 mg of regular caffeine, but no changes were observed following doses of 100 and 200 mg. Others have reported an increase in blood pressure following a 4 $mg \cdot kg^{-1}$ dose of regular caffeine with breakfast (Smith et al., 1994). In contrast, elevated blood pressure and a decrease in heart rate has also been reported following 120 mg of regular caffeine (Mitchell et al., 2011).

This appears to be the first study to examine the effects of a time-release caffeine containing supplement on metabolic measures and a marker of lipolysis. Both CAF and TR-CAF supplements administered during this study did not alter metabolic measures or glycerol concentrations. Previous investigations have shown that ingestion of caffeine can stimulate an increase in metabolic rate in a dose dependent manner (Acheson et al., 1980; Astrup et al., 1990; Dulloo et al., 1989). However, these results have not been consistent. Acheson and colleagues (1980) have reported that 4 $mg \cdot kg^{-1}$ of regular caffeine can significantly elevate resting energy expenditure and fat oxidation with and without a meal, while Astrup et al. (1990)

showed that a 400 mg dose of regular release caffeine can significantly elevate resting energy expenditure for 3 hours post-ingestion. Interestingly, those investigators did not see the same effect when a 200 mg dose was ingested. However, others have reported significant increases in resting energy expenditure with as little as 100 mg of regular release caffeine (Dulloo et al., 1989; Hollands et al., 1981; Koot and Deurenberg, 1995). Contrary to our findings, Astrup et al. (1990) showed significantly greater elevations in glycerol concentration following 100, 200, and 400 mg of regular caffeine compared to placebo. Acute doses of 330 mg (Costill et al., 1977) and 6 $mg \cdot kg^{-1}$ (Ryu et al., 2001) of regular caffeine have also shown to increase energy expenditure along with glycerol concentrations during steady-state exercise. It is possible that the relatively sedentary activity performed by the participants during the current study period may have negated any potential benefit in regards to energy expenditure.

Caffeine has been associated with enhancing the ability to perform mental tasks and elevate feelings of energy in low to moderate caffeine consumers (Christopher et al., 2005; Lieberman, 2001; Maridakis et al., 2009; Maridakis et al., 2009; Smith, 2005; Warburton et al., 2001). However, in the current study, CAF and TR-CAF did not have any effect on mood states (POMS) or subjective feelings of energy, alertness, or focus during the 8 hour study. Penetar et al. (1993) reported that sleep deprived caffeine users significantly elevated alertness and improved feelings of vigor, fatigue, and confusion for up to 2 hours following the ingestion of 2.1, 4.3, or 8.6 $mg \cdot kg^{-1}$ of regular caffeine. The differences between this study and others may be related to the absorption rate and absolute peak caffeine concentration in plasma. Penetar and colleagues reported that the participants in their study reached peak plasma caffeine concentrations of 2.6, 5.9, and 12.3 $mg \cdot L^{-1}$, respectively, which were all greater that that seen in this study. Improvements in mood states and subjective measures of energy, focus, and alertness have also been observed following the ingestion of time-release caffeine at larger doses. Following sleep deprivation, 300 to 600 mg of time-release caffeine has been shown to improve subjective measures of sleepiness (Beaumont et al., 2004), vigor, fatigue (De Valck et al., 2003), and attentiveness (Lagarde et al., 2000) for up to 9 hours. The lower dose administered in the current study did not appear to provide a sufficient stimulus in habitual caffeine consumers.

In the current study, the average physical reaction time, measured as the total elapsed time for the participant to identify a target stimulus, physically touch the stimulus with their hand, and return the hand back to a home button, was significantly faster at hour 5 following CAF ingestion compared to PL. Additionally, average upper body reaction time, measured as the number of successful "hits" in 60 seconds, was significantly improved for CAF and TR-CAF during hours 1 - 4 and over the 8 hour study duration. Interestingly, only TR-CAF had an average upper body reaction time significantly better than PL during hours 5 - 8, potentially as a result of the slower rate of caffeine absorption. Previous research has reported that time-release caffeine is effective for maintaining

vigilance and cognitive function following sleep deprivation for up to 13 hours using a single 300 mg dose (De Valck et al., 2003; Doireau et al., 1997; Lagarde et al., 2000), a single 600 mg dose (De Valck and Cluydts, 2001; Lagarde et al., 2000; Patat et al., 2000), and two daily 300 mg doses (Beaumont et al., 2005; Beaumont et al., 2001). In contrast to these previous studies, the current study utilized a multi-ingredient supplement containing a lower dose (194 mg) of time-release caffeine in habitual caffeine consumers without sleep deprivation. It is possible that the low dose in relatively rested participants did not provide a sufficient stimulus to benefit other performance measures including lower body reaction time, multiple object tracking, and cognition. It has been suggested that plasma caffeine concentrations need to exceed 2.5 mg·L^{-1} to stimulate performance effects (Beaumont et al., 2001; 2004; 2005). The plasma caffeine concentrations in the current study did not exceed 2.5 mg·L^{-1} during the 8-hour study, which may potentially explain the lack of improvement seen compared to PL in many of the performance measures. However, improvements in cognitive function and vigilance have also been observed following as little as 12.5 (Smit and Rogers, 2000) and 32 mg ingestion of regular caffeine (Lieberman et al., 1987). Nevertheless, the delayed absorption of a time-release formulation may require a greater caffeine intake. Lagarde et al. (2000) compared time-release caffeine doses of 150, 300, and 600 mg in sleep deprived subjects. Their findings indicated that the optimal dose for improving vigilance and cognitive function during a 13-hour study, without any side effects, was 300 mg. Thus, the 194 mg dose in TR-CAF does not appear to provide a sufficient increase in plasma caffeine concentrations to elicit positive effects on cognitive function in regular caffeine consumers.

In the current study, we acknowledge potential limitations when comparing the effects of TR-CAF and CAF. The TR-CAF supplement included other ingredients with relatively mild stimulatory effects (i.e., theobromine, rhodiola-rosea, and ginseng), while CAF contained only the equivalent amount of caffeine. It is possible that the other ingredients interfered with metabolic measures, cardiovascular measures, and subjective measures, however both supplements yielded very modest effects. These methods allowed the researchers to compare the time-release caffeine containing supplement with a caffeine bolus as it is typically ingested by habitual caffeine users.

Conclusion

The multi-ingredient supplement, TR-CAF, and CAF showed distinct caffeine pharmacokinetic differences yielding modest effects on reaction time, yet did not alter glycerol concentrations, metabolic measures, cardiovascular measures, or subjective measures of mood states in habitual caffeine consumers. Also, a single dose of 194 mg of caffeine either in TR-CAF or CAF did not affect lower body reaction time, multiple object tracking, or cognitive function. Although caffeine has previously shown to increase the ability to perform mental tasks and

elevate feelings of energy, the lower dose administered in the current study did not appear to be sufficient to stimulate similar effects in habitual caffeine consumers.

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Key points

- Time-release caffeine and regular caffeine showed distinct pharmacokinetics over an 8-hour period following ingestion.
- Time-release caffeine and regular caffeine yielded modest effects on reaction time over an 8-hour period following ingestion.
- Time-release caffeine and regular caffeine did not alter glycerol concentration, metabolic measures, or other performance measures over an 8-hour period following ingestion.

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