Title: Caffeine consumption around an exercise bout: effects on energy expenditure, energy intake, and exercise enjoyment

Running head: Effects of caffeine and exercise on acute energy balance

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Abstract

Combining an exercise and nutritional intervention is arguably the optimal method of creating energy imbalance for weight loss. This study sought to determine if combining exercise and caffeine supplementation was more effective for promoting acute energy deficits and manipulations to substrate metabolism than exercise alone. Fourteen recreationally-active participants (Mean ± SD BMI: 22.7 ± 2.6 $kg \cdot m^{-2}$) completed a resting control trial (CON), a placebo exercise trial (EX), and a caffeine exercise trial (EX+CAF, 2x 3 $mg \cdot kg^{-1}$ of caffeine 90 min before and 30 min after exercise) in a randomized, double-blinded design. Trials were 4 h in duration with 1 h of rest, 1 h of cycling at ~65 % power at $\dot{V}O_2max$ or rest, and a 2 h recovery. Gas exchange, appetite perceptions, and blood samples were obtained periodically. Two hours after exercise, participants were offered an *ad libitum* test meal where energy and macronutrient intake were recorded. EX+CAF resulted in significantly greater energy expenditure and fat oxidation compared to EX (+250 kJ; +10.4 g) and CON (+3126 kJ; +29.7 g) ($P < 0.05$). A trend for reduced energy and fat intake compared to CON (-718 kJ; -8 g) ($P = 0.055$) was observed. Consequently, EX+CAF created a greater energy deficit ($P < 0.05$). Caffeine also led to exercise being perceived as less difficult and more enjoyable ($P < 0.05$). Combining caffeine with exercise creates a greater acute energy deficit and the implications of this protocol for weight loss or maintenance over longer periods of time in overweight/obese populations should be further investigated.

Keywords: exercise; appetite; caffeine; fat oxidation; exercise enjoyment

Introduction

Energy balance is an important concept in weight and obesity management, as understanding and manipulating energy balance can lead to changes in body composition or body weight. Exercise is consistently recommended for weight loss, weight maintenance, and improving general and metabolic health (18). Recent systematic reviews have indicated that exercise causes minimal compensatory behavior in most healthy individuals in regard to energy intake and non-exercise energy expenditure both acutely (< 24 h) and over weeks and months (19, 41, 50).

Caffeine is the most widely consumed psychoactive substance in the world, with a recent study reporting 85% of a sample population (~38,000) consumed one or more caffeine-containing beverages a day (36). Thus, it is important and practical to examine what influence caffeine has on determinants of energy balance. There is strong evidence that caffeine can lead to subtle increases in resting energy expenditure (~5 % over 24 h) (28). However, caffeine's effects on appetite and energy intake are more variable, with some studies reporting reductions in energy intake (47), while others do not (23, 24, 26); however, these studies were all conducted in resting conditions. The mechanisms by which caffeine may influence appetite and energy intake remain unknown, but may be related to adenosine antagonism or changes in neurotransmitters such as dopamine (22). Although most of these studies did not incorporate measures of energy expenditure, alterations in resting metabolism, such as increased heart rate, fat oxidation, and skin temperature have been documented with caffeine consumption (1, 3).

A pair of studies examined the effect of 4 days of caffeine supplementation on spontaneous activity and energy expenditure, but observed no changes in these variables using a variety of assessment methods (31, 32). While novel, these two studies were focused on free-living energy expenditure and non-exercise physical activity. The effect of caffeine supplementation on a structured exercise bout and the potential alterations in energy balance remain to be elucidated. Caffeine may also alter behavior via increasing enjoyment of physical activity (40), which could be due to aforementioned changes in neurotransmitters and have implications for longer-term exercise participation. This makes caffeine of particular interest as a potential nutritional intervention to utilize in combination with exercise to manipulate energy balance. However, to our knowledge, no study has simultaneously examined how caffeine influences energy expenditure and energy intake in congruence with exercise.

Given the widespread habitual consumption of caffeine and the recommendations of exercise participation for health and weight loss and maintenance, examining the interactions of caffeine and exercise from an energy balance perspective may provide a highly practical strategy for creating acute and chronic energy deficits. Thus, the aim of the present study was to examine

how caffeine ingestion influences energy balance around an acute bout of exercise in recreationally-active men and women. We hypothesized that caffeine supplementation with exercise would lead to higher energy expenditure and fat oxidation without alterations or compensation in energy intake, thereby creating a larger energy deficit than exercise alone.

Methods

Overview

This study was a double-blind, randomized, placebo-controlled crossover trial conducted according to the guidelines in the Declaration of Helsinki. All procedures were approved by the Griffith University Human Research Ethics Committee (GU protocol: PBH/52/13/HREC) and all participants provided written informed consent before enrolment. Participants completed 3 experimental conditions a minimum of 3 d and maximum of 1 month apart in a random order: a resting control trial (CON), a placebo exercise trial (EX), and a caffeine-exercise trial (EX+CAF). A randomization sequence was generated using randomization software (http://www.randomizer.org/) for each participant. An individual not associated with data collection further randomized participants to caffeine or placebo treatment for each exercise trial. Procedures were identical for all trials with the exception that no exercise was performed during the CON trial. Participants were informed the purpose of the study was to examine the effects of exercise and caffeine on energy balance, and that energy expenditure would be measured during the laboratory visits. At the end of each visit, they were told that they would be given a free lunch. They were not informed this lunch was being measured until debriefing upon completion of the study.

Familiarization and screening

Participants were required to be non-smoking; non-obese (BMI < 30 $kg \bullet m^{-2}$), pre-menopausal (women), between 18 and 45 years of age; not taking any medicine known to influence lipid, carbohydrate, or caffeine metabolism (except oral contraceptives); not dieting; weight stable in the previous 3 months (± 5 % by self-report); no history of any cardiovascular or metabolic diseases; no food allergies or intolerances; no history of gastrointestinal disorders; and recreationally active (defined as ≥ 30 min of moderate intensity exercise/d, ≥ 3 d/wk). Participants were recruited by e-mail advertisements through the university e-mail system. All participants completed preliminary paperwork on health, exercise, and dietary behaviors before attending any laboratory sessions.

Dietary restraint, dietary disinhibition, and susceptibility to hunger were assessed using the Three-Factor Eating Questionnaire (TFEQ) (46). Participants were excluded if they exceeded the cut-offs of 12, 9, 8 for restraint, disinhibition, and hunger, respectively. Dietary caffeine intake was assessed using a caffeine-consumption questionnaire to quantify daily consumption (13).

Participants completed a familiarization and screening visit for determination of body composition, $\dot{V}O_2max$, and to be familiarized with the computer questionnaires utilized in the study. Informed consent was also obtained at this time. Height and weight were determined for calculation of body mass index. Body density was determined via the sum of three skinfolds specific for sex using established equations (29, 30). Percent body fat was calculated from body density utilizing the Siri equation (43). Participants then mounted a cycle ergometer (Lode Excalibur Sport; Lode B.V., Groningen, N.L.) and initiated exercise at 30 W for 3 min after which workload was increased by 20 $W \cdot min^{-1}$ (women) or 30 $W \cdot min^{-1}$ (men) following a ramp protocol until volitional fatigue (cadence < 50 $rev \cdot min^{-1}$) was attained. Pedal cadence was maintained at 60 – 90 $rev \cdot min^{-1}$, and participants were encouraged to exercise "all-out". Heart rate (Polar Electro, Kempele, Finland), $\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}_E$, and RER (Quark C-PET; Cosmed, Italy) were monitored continuously. $\dot{V}O_2max$ attainment was confirmed with the following criteria: RER $\geq$ 1.10, heart rate $\pm$10 $b \cdot min^{-1}$ of age-predicted maximum, and/or a plateau in $\dot{V}O_2$ < 2.0 $mL \cdot kg^{-1} \cdot min^{-1}$ (39).

Standardization of diet and exercise

Before all experimental trials, participants were instructed to refrain from alcohol and caffeine intake for 24 h. Also in the 24 h before each trial, participants were asked to complete a food diary and record all food and drink. This diary was photocopied and returned to the participants who were asked to replicate their intake for all subsequent trials. Food intake the 24 h before each trial was examined using dietary analysis software (FoodWorks®, Xyris Software; Kenmore Hills, Australia). Participants were also asked to refrain from strenuous exercise the 18 hours before their trial, and this was confirmed through verbal compliance at the start of each trial.

Experimental trials

A timeline of the experimental trials is displayed in **Figure 1**. Participants arrived to the laboratory in the morning (06.00-09.00 h) after an overnight fast (10-12 h), having minimized physical activity en route. Upon arrival, participants completed visual analogue scales for measures of mood, physical symptoms, and appetite while lying in a semi-supine position. Simultaneously, a

cannula was inserted into an antecubital vein for blood sample collection. Also at this time (during the exercise trials), 3 mg·kg^{-1} BM of caffeine (PCCA, Matraville, NSW, Australia) or a placebo (Metamucil®) was ingested with 250 ml of water. Our group has used this compound before as a texture-matched placebo with no reported issues and the small amount of fiber (~400 mg) likely had no influence on the variables examined. After giving participants ~20 minutes to rest quietly and acclimate, the participants began the first resting period of 60 min. Resting energy expenditure (REE) was measured continuously for the final 20 min of this period using indirect calorimetry (Quark C-PET; Cosmed, Italy) and a facemask (V2 Mask; Hans-Rudolph, Inc., USA) with the participants in a semi-supine position, using suggested best-practice methods (9). Coefficients of variation in our lab for REE, $\dot{V}O_2$, and $\dot{V}CO_2$ are 5.2 %, 4.8 %, and 5.6 %, respectively.

Participants then completed 60 min of exercise on a cycle ergometer, at ~65 % power output of $\dot{V}O_2$max (EX and EX+CAF) or rested/worked quietly (CON). During exercise, mechanical work and power output data were recorded continuously, while gas exchange ($\dot{V}O_2$, $\dot{V}CO_2$, RER) was recorded for the first 15 min and then from 25-35 min and 45-55 min. Power output was adjusted as necessary to maintain ~65% of the estimated power output at $\dot{V}O_2$max. The attained power output from the first exercise trial was duplicated in the second one. At 15 min intervals, participants provided ratings of perceived exertion (6), leg pain (10), and pleasure/displeasure (Feeling Scale) (27); heart rate was also recorded at these times. Enjoyment was assessed post-exercise via completion of the Physical Activity Enjoyment Scale (PACES) (33). During the equivalent period of the CON trial, gas exchange data were collected over the final 15 min to estimate energy expenditure; no perceptual measures were taken.

Post-exercise, gas exchange was measured throughout the final 15 minutes of each subsequent hour (+165-180 and +225-240 min). At +150 min (30 min post-exercise/rest), participants were given another 3 mg·kg^{-1} BM dose of caffeine or placebo along with a small liquid calorie meal (250 mL, 825 kJ, 30.3 g carbohydrate, 3.8 g fat, 8.3 g protein; Up N Go®, Sanitarium Health & Well-Being™; Australia). This beverage was provided to provide participants with a small amount of calories and examine post-prandial responses during all trials over the final 90 min. After the measurement period concluded (+240 min), the cannula was removed and participants were offered a lunch meal for the determination of single meal *ad libitum* energy intake. All trials were conducted in a thermoneutral laboratory environment (Mean±SD: 23.3±0.5 °C, 70.5±7.0 % relative humidity, and 758.4±3.0 mmHg). Heart rate was recorded throughout the trials using short-range telemetry (Polar Electro).

The rationale for dividing the caffeine dosage was threefold. First, 3 mg·kg^{-1} BM has been shown to improve exercise performance to the same degree as larger doses (15). Second, by using

multiple doses several hours apart, typical patterns of caffeine/coffee consumption are mirrored (i.e. early and mid-morning coffee). A recent study reported that coffee consumption peaks in the morning (0600-0800) with a second peak late morning (1000-1200), with a decline thereafter during the week (25). The third reason was to maintain plasma caffeine levels in order to examination their relationship to other variables.

Appetite perceptions

Starting at baseline and every 30 min throughout the trials except for during the 60 min of exercise or rest, participants completed visual analogue scales on a computer for appetite perceptions (hunger, fullness, satisfaction, desire to eat). After the lunch meal, ratings of pleasantness, palatability, and acceptability of the meal were also determined (Adaptive VAS; San Antonio, USA).

Blood sampling

Blood samples (8 total) were taken at baseline (-30 min), 15 min, pre-exercise (60 min), post-exercise (120 min), 150 min, 180 min, 210 min, and pre-lunch (240 min). At each sample point, 3 mL of blood was collected into sodium fluoride tubes (BD Vacutainer; North Ryde, Australia) for determination of plasma glucose and lactate and 4 mL into EDTA tubes (BD Vacutainer) for determination of plasma triglycerides. At baseline (-30 min), 15 min, pre-exercise (60 min), 180 min, and 240 min, an additional 3 mL of blood was collected into lithium heparin tubes (BD Vacutainer) for determination of plasma caffeine and paraxanthine (exercise trials; a baseline-only sample was collected during CON to ensure compliance regarding abstinence from caffeine intake). All samples were centrifuged at 10°C for 15 min at ~1000 *g* with the plasma stored at -80°C until analysis. Samples for plasma glucose (GLUC HK Gen. 3), lactate (LACT Gen. 2), and triglycerides (TRIGL) were analyzed in duplicate on a COBAS Integra 400 *plus* diagnostic system (Roche Diagnostics, Switzerland). Plasma caffeine and paraxanthine were determined using HPLC (14) in triplicate with standards of known concentration. Samples from each participant were analyzed within the same run to remove interassay variation. The intra-assay coefficients of variation were 3.8 % for glucose, 3.6 % for lactate, 4.0 % for TGs, 5.2 % for caffeine, and 6.7 % for paraxanthine. Blood samples were not corrected for plasma volume as exercise of comparable or greater duration and intensity has previously been shown not to result in acute plasma volume changes (7).

Fluid and ad libitum energy intake

Fluid intake was standardized between trials. Participants were given 250 mL of water upon arrival. A further 500 mL of water was provided during the exercise and rest (control) period in two 250 mL servings (at 15 and 45 min) and another 250 mL serve between end-exercise and lunch (+180 min). If participants did not consume all fluid offered in their first exercise trial, the amount consumed was replicated for the second one.

Two hours after exercise (240 min), participants were provided with an *ad libitum* meal for assessment of energy intake (EI) and instructed to eat until satisfied. Participants' were given their choice of a Subway® sandwich and toppings (kept consistent between trials; sandwich and topping weights were not different, 545±90 g, $P = 0.632$) and offered a number of side items including savory, salty, and sweet foods which may form part of a typical lunch meal (i.e. potato chips, apple, banana, and cookies). The sandwich portion of the meal was always fixed at one 'foot-long' sandwich (12 in; 30.5 cm), but more side items were available if desired. Participants consumed their meals separately from the investigator or any other participants in a quiet environment. All food was weighed beforehand and presented in excess of expected consumption. Sandwiches were weighed as complete items. Food was weighed again after participants left the laboratory, and the manufacturers' values and a nutrition software database (FoodWorks®, Xyris Software; Kenmore Hills, Australia) were used to calculate energy and macronutrient intake.

Calculation of energy expenditure, substrate oxidation, and compensation

Gas exchange data, obtained via indirect calorimetry, was averaged in 30-sec segments over the final 5 minutes of each 10-20 min collection period. Energy expenditure was quantified in the following increments: resting period (0-60 min), exercise/control period (60-120 min), post-exercise recovery period 1 (120-180 min), recovery period 2 (180-240 min), and total trial (0-240 min). Rates and total substrate oxidation were calculated for identical time periods using the equations of Frayn (21) and assuming negligible protein oxidation. Energy equivalents of 16.75 $kJ \cdot g^{-1}$ of carbohydrate and 37.68 $kJ \cdot g^{-1}$ of fat were utilized to calculate energy expenditure from substrate oxidation.

Estimated relative energy intake (eREI) was calculated by subtracting energy expenditure from the energy intake of each trial. Post-exercise energy compensation (PEEC) was calculated per the suggestion of Cadiuex and colleagues (8), as:

$$\left([(EIexer - EIctrl) + (EEctrl - EEexer)] / ExEE\right)*100$$

Where EIexer is energy intake of the EX trials, EIctrl is energy intake of the CON trial, EEexer is total energy expenditure of the EX session, EEctrl is total energy expenditure of the CON session, and ExEE is the energy expenditure of the EX bout. Values below 100% would suggest incomplete compensation, while values greater than 100% suggest overcompensation.

Data analysis & statistics

Power calculations performed *a priori* suggested a minimum of 12 participants was necessary to detect a 500 kJ difference in *ad libitum* energy intake between treatments, with an estimated standard deviation of 1000 kJ, at a power level of 80%.

Data were analyzed in an appropriate software package (SPSS v. 22, Chicago, USA). Normality was tested using the Shapiro-Wilk test. Differences between men and women for demographic data were assessed using independent samples *t*-tests. One-way repeated measures ANOVA explored differences between trials for total energy expenditure, substrate oxidation, energy and macronutrient intake, eREI, and area under the concentration-time curve (AUCs) for appetite perceptions and blood variables. Repeated-measures, two-factor (trial*time) ANOVA examined changes between trials over time with the Bonferroni *post-hoc* adjustment for multiple comparisons. The main aim of this study was not to examine differences between sexes or habitual caffeine intake, and the study lacked the power to do so. Mean differences between bouts of exercise and PEEC were examined with paired *t*-tests. Data in text and tables are presented as means ± SD whilst data in figures are presented as means ± SEM for clarity and to avoid distortion. AUCs were calculated using the trapezoidal rule. Relationships between variables were examined using Pearson and partial correlations. Effect sizes for relevant comparisons (total energy expenditure, energy intake, relative energy intake, and exercise parameters) were calculated using Cohen's *d*, and defined as trivial (< 0.20), small (0.20-0.40), moderate (0.40-0.80), and large (> 0.8), respectively. Differences were accepted as significant if $P < 0.05$ and all *P*-values have already been adjusted for multiple comparisons.

Results

Twenty-four participants were initially recruited and screened for the study. Seven participants failed to meet inclusion criteria (exhibited clinical levels of dietary restraint and disinhibition or medication usage). Seventeen completed screening and familiarization testing, but contact with one male participant was lost between screening and data collection. Another participant completed her first trial but withdrew thereafter due to a family emergency. Fifteen

participants (8 women (3 using oral contraceptives), 7 men) completed all phases of the study. During data analysis, one male participant was discovered to have started all three of his trials with elevated plasma caffeine levels (44 SD above mean values). Therefore this participant has been excluded from analyses due to his non-compliance. Demographics of the remaining study participants (n = 14) are displayed in **Table 1**. Habitual caffeine intake was similar between sexes ($P = 0.925$), but due to differences in body weight, the men received a significantly greater dosage during the experimental trials ($P < 0.005$). Where possible, women completed all trials between days 1-14 of their menstrual cycle (n = 4); when this was not possible, two trials were completed during the first phase and the final trial during the same phase 3-4 weeks later (n = 4). The majority of participants (n = 12) habitually exercised in the morning and in a fasted state. Dietary intake in the 24 h before each trial was well matched (CON: 8.6±2.7 MJ, EX: 8.3±2.7 MJ, EX+CAF: 8.5±2.6 MJ; $P = 0.972$). No order effects were observed during the trials for energy expenditure, energy intake, appetite, substrate oxidation, or plasma metabolites ($P > 0.05$ for all).

Six participants had no idea or incorrectly guessed which trial they had received caffeine. Four participants correctly identified their caffeine trials, but only had "some idea" of their certainty of having received caffeine. Three participants correctly reported they were "pretty sure" they had received caffeine. Finally, one participant correctly identified his caffeine trial and was "absolutely certain" he had received caffeine.

Energy expenditure and substrate oxidation

Energy expenditure and substrate oxidation for each measurement period and total trial are shown in **Table 2**. Two-factor repeated-measures ANOVA revealed main effects of trial ($P < 0.001$), time ($P < 0.001$), and a significant trial*time interaction ($P < 0.001$) for energy expenditure. Energy expenditure was higher during the exercise period and also the two hours post-exercise compared with the resting period ($P < 0.01$ for all). Furthermore, energy expenditure was higher during the resting period, exercise period, and the second post-exercise recovery period in EX+CAF compared to CON and EX ($d = 0.25 – 1.05$). Total energy expenditure was significantly higher in EX+CAF compared to EX ($d = 0.41$) and CON ($d = 5.18$), while energy expenditure during EX was also higher compared to CON ($d = 5.09$) ($P < 0.001$ for all).

Regarding carbohydrate oxidation, a main effect of trial ($P < 0.001$), time ($P < 0.001$), and a trial*time interaction ($P < 0.001$) were revealed. Mean CHO oxidation was significantly elevated in EX and EX+CAF compared to CON ($P < 0.01$ for both; $d = 5.04$ and 4.94); these results were supported by the results for total CHO oxidation. CHO oxidation was significantly elevated during

the exercise period compared to all other time points ($P < 0.01$ for all); CHO oxidation was also elevated in the final hour compared to rest and the first hour post-exercise ($P < 0.001$ for both).

For fat oxidation, a main effect of trial ($P < 0.001$), time ($P < 0.001$), and a trial*time interaction ($P < 0.001$) were observed. Mean fat oxidation was significantly greater for EX+CAF compared to CON ($P < 0.001$) and EX ($P = 0.005$) and was also higher for EX compared to CON ($P < 0.001$); this was supported by total fat oxidation results which showed that EX+CAF was greater than EX ($d = 0.98$; $P = 0.005$) with both having greater fat oxidation than CON ($d = 2.94$ & 2.51; $P < 0.001$ for both). Fat oxidation was elevated at rest, during exercise, and during the second hour of recovery in EX+CAF compared to EX and CON ($d = 0.75$-1.21; $P < 0.05$ for all). EX+CAF and EX fat oxidation were also elevated during the first hour of recovery compared to CON ($P < 0.05$).

Energy intake, relative energy intake and post-exercise energy compensation

EI and macronutrient intake at the *ad libitum* lunch meal, eREI, and total weight of the meal consumed are displayed in **Table 3**. Individual data for EI and eREI are displayed in **Figure 2**. Eight participants (4 men and 4 women) ate less in EX+CAF compared to the other trials, 2 women consumed the least in CON, 2 participants (1 man and 1 woman) consumed the least in EX, and 2 participants (1 man, 1 woman) consumed similar amounts between trials.

One-way repeated-measures ANOVA revealed a significant difference between trials for EI ($P = 0.036$). EI trended towards being lower in EX+CAF compared to CON ($d = 0.51$; $P = 0.055$) but was not different for EX+CAF compared to EX ($d = 0.44$; $P = 0.109$) (**Table 3**). CHO ($P = 0.147$) and PRO ($P = 0.344$) intake were not significantly different between trials. A significant difference between trials was observed for fat intake ($P = 0.031$); fat intake was significantly decreased in EX+CAF compared to CON ($P = 0.04$) and tended to be decreased compared to EX ($P = 0.06$). Meal weights were significantly different between trials ($P = 0.032$), with EX+CAF significantly less than CON ($d = 0.42$; $P = 0.05$), but *post-hoc* comparisons between EX+CAF and EX ($d = 0.42$; $P = 0.102$) failed to reach significance.

Estimated REI was significantly different between trials, with the greatest energy deficit in the EX+CAF condition compared to EX ($d = 0.71$; $P = 0.033$) and CON ($d = 3.0$; $P < 0.001$). EX also had a greater deficit than CON ($d = 2.08$; $P < 0.001$). Despite the differences in eREI, PEEC did not reach significance between the exercise trials (Paired-samples *t*-test; $P = 0.110$; $d = 0.41$). Participants compensated for 85±36 % of total EE in the EX trial and 71±32 % of total EE in the EX+CAF trial.

Appetite perceptions

Profiles for hunger and satisfaction are displayed in **Figure 3**. Baseline appetite perceptions were not significantly different between the trials ($P > 0.3$). Two-factor repeated-measures ANOVA revealed a main effect of time ($P < 0.001$), a significant trial*time interaction ($P = 0.038$), but no effect of trial ($P = 0.520$) for hunger ratings during the trials. Hunger was higher in CON than EX and EX+CAF at 30, 60, and 120 min ($P < 0.05$), whilst it was also higher in CON compared with EX+CAF at 150 min ($P < 0.05$) (**Figure 3**). For satisfaction, a main effect of time ($P = 0.003$) and a time*trial interaction were also observed ($P = 0.002$), but no effect of trial was detected ($P = 0.662$). Ratings of satisfaction were higher at 120 and 150 min in EX and EX+CAF compared to CON ($P < 0.05$) (**Figure 3**). Profiles for desire to eat and fullness showed similar effects (data not shown).

Total AUC for hunger was significantly different between trials ($P = 0.03$), with CON higher than EX+CAF ($P = 0.034$). No other significant differences were observed in total appetite AUCs between trials.

Overall acceptance (palatability) of the meal was not significantly different between trials (CON: 80.4±12.9, EX: 80.4 ±12.6, EX+CAF 80.5±13.1; $P = 0.999$).

Plasma metabolites

Owing to difficulties with blood collection, complete blood sample data were available for 8 participants (5 men; 3 women). Profiles for glucose, lactate, and triglycerides are displayed in **Figure 4**.

Baseline values for glucose ($P = 0.341$), lactate ($P = 0.384$), and triglycerides ($P = 0.66$) were not significantly different between trials. Results showed a main effect of time ($P < 0.001$) but no effect of trial ($P = 0.097$) or time*trial interaction ($P = 0.413$) for plasma glucose (**Figure 4).** Glucose levels at 180 min were significantly elevated compared to all other time points (first time point post-drink ingestion). Plasma lactate results revealed significant main effects for time ($P < 0.001$), trial ($P = 0.005$), and a significant time*trial interaction ($P = 0.014$) (**Figure 4**). Lactate levels were significantly elevated at 120 min (post-exercise) for EX ($P = 0.004$) and EX+CAF ($P = 0.013$) compared to CON. For plasma triglycerides, a main effect of time ($P < 0.001$) and a significant time*trial interaction ($P = 0.008$), but no main effect of trial ($P = 0.051$) (**Figure 4**) were observed. Triglycerides were significantly elevated in the EX+CAF trial compared to CON at 60,

120, 210, and 240 min ($P < 0.05$) and also elevated above EX at 120 and 210 min ($P < 0.05$). During EX, triglycerides were elevated above CON at 120 and 240 min ($P < 0.05$).

Baseline values for caffeine and paraxanthine were not significantly different between trials ($P > 0.4$) and were < 1.5 μmol·L^{-1} for caffeine, suggesting compliance with pre-trial guidelines. In the EX+CAF trial, caffeine levels peaked by 60 min (~90 min post-treatment) at ~21 μmol·L^{-1} and were stable until increasing again between 180-240 min (30-90 min post-second treatment ingestion), reaching a peak of ~33 μmol·L^{-1} by 240 min. Paraxanthine levels rose continuously throughout the EX+CAF trial reaching a peak concentration of ~8.1 μmol·L^{-1} at 240 min.

Other exercise responses

Exercise intensity (EX: 68.3±5.5, EX+CAF: 68.4±3.7 % $\dot{V}O_2$max; $P = 0.92$), oxygen consumption (EX: 2.18±0.40, EX+CAF: 2.21±0.44 L·min^{-1}; $P = 0.478$), mechanical work (EX: 436±85, EX+CAF: 437±82 kJ; $P = 0.714$), and heart rate (EX: 145±18, EX+CAF 148±14 bpm; $P = 0.218$) were well-matched between exercise trials. $\dot{V}_E$ was increased ($P = 0.002$; $d = 0.35$) during EX+CAF (58.1±9.5 L·min^{-1}) compared to EX (54.7±10.5 L·min^{-1}). Exercise during the EX+CAF trial was perceived as less difficult (RPE EX: 14±2, EX+CAF: 13±2; $P = 0.028$, $d = 0.52$) and more enjoyable (PACES EX: 87±17, EX+CAF: 97±18; $P = 0.019$, $d = 0.58$), but pain (EX: 3.2±2, EX+CAF: 2.6±2; $P = 0.092$, $d = 0.31$) and pleasure/displeasure (FS EX: 1.9±2, EX+CAF: 2.4±2; $P = 0.104$, $d = 0.26$) were not significantly different.

Relationships between variables

Correlational analyses revealed a number of relationships between variables. Pearson correlation analysis revealed a modest trend between fat oxidation and total triglyceride AUC ($r = 0.394$; $P = 0.057$), but this effect disappeared when controlling for trial (partial $r = 0.138$; $P = 0.531$). During the exercise bouts, we observed significant relationships between ventilation and energy expenditure (partial r controlling for trial = 0.841, $P < 0.001$), fat oxidation and energy expenditure (partial $r = 0.495$, $P = 0.009$), and a trend for a relationship between ventilation and fat oxidation during exercise (partial $r = 0.350$, $P = 0.074$). In addition, measures of affect (pleasure/displeasure) were correlated with absolute energy intake (partial $r = -0.436$, $P = 0.023$) while enjoyment of physical activity was correlated with estimated REI (partial $r = -0.411$, $P = 0.033$). Finally, caffeine and paraxanthine AUCs were correlated with eREI ($r = -0.518$ and -0.47, respectively; $P < 0.025$ for both), total fat oxidation ($r = 0.784$ and 0.694; $P < 0.001$ for both), and

total energy expenditure (r = 0.537 and 0.48; $P < 0.022$ for both); however, none of these correlations remained significant after controlling for trial.

Discussion

The present study aimed to examine the ability of caffeine supplementation to acutely manipulate energy expenditure, substrate metabolism, and energy intake in combination with a bout of moderate exercise in active individuals. The primary findings were that caffeine and exercise together led to significantly greater fat oxidation and energy expenditure, and a larger energy deficit compared to exercise alone. Additionally, EX+CAF was also associated with changes to a number of perceptual variables during and after the exercise bout that may have beneficial implications for exercise enjoyment and participation.

A number of studies have demonstrated caffeine's ability to increase resting energy expenditure and metabolism (1, 3, 28), which is likely mediated via activation of the sympathetic nervous system (SNS) (1). Our results confirm these findings, as the EX+CAF trial had a higher EE in the time periods after caffeine ingestion (0-60 and 180-240 min). In addition, exercise energy expenditure was also significantly greater in EX+CAF. Caffeine's ability to influence substrate metabolism, energy expenditure, ventilation, and oxygen consumption has been previously established at rest in men and women (2, 3) and during moderate intensity exercise (~55 % $\dot{V}O_2max$/65 % HR_{max}) in untrained men and women (17, 49); we extend these findings by establishing that caffeine before and after exercise, in a pattern of typical consumption, can increase energy expenditure and fat metabolism in recreationally active men and women before, during, and after moderate exercise. The implications of these differences in EE, and the potential time course that an increased EE would be sustained, require further investigation. It would also be interesting to compare habitual and non-habitual caffeine consumers, since non-habitual consumers may be more sensitive to the thermogenic effects of caffeine (51). Habituation to caffeine and any changes in its effectiveness would also be worth investigating to see if the development of tolerance blunts changes in EE.

Regarding the closest available literature on caffeine supplementation and energy balance, a pair of studies revealed that caffeine supplementation (5 $mg \cdot kg^{-1}$ BM per d in two 2.5 $mg \cdot kg^{-1}$ doses) led to non-significant decreases in energy intake (-490 kJ and -880 kJ) and a trend for decreased fat intake in the second study (-13 g; P = 0.052) over 4 d of supplementation (31, 32). Energy intake was assessed each day with 24 h food diaries and averaged for each 4 d condition (caffeine or placebo). However, these authors observed no changes in physical activity, non-

exercise physical activity, total energy expenditure, steps per day, or REE during caffeine supplementation (31, 32). The main explanation for the difference in energy expenditure results observed in the present study with caffeine supplementation and the results of Judice and colleagues could be that we utilized a structured, prolonged, strenuous exercise bout in addition to caffeine to manipulate energy expenditure (31, 32); therefore, caffeine and exercise synergistically manipulating the SNS may have had an additive effect on EE and EI.

Individuals are not driven to compensate for energy expended during exercise in the immediate hours (< 12 h) after a single bout (19, 41). Our current study supports these findings by revealing that participants only incompletely compensated for the exercise-induced energy deficit. Interestingly, a greater deficit was noticeable for EX+CAF compared to EX and CON and this appeared to be at least partially mediated by decreased fat consumption and increased fat oxidation. A study by Tremblay and colleagues previously observed decreased energy and fat intake in men at a test meal 30 min after ingesting 300 mg of caffeine compared to placebo (47), but two other studies assessing acute effects of caffeine on single-meal energy intake did not achieve this result when using a single-item meal, which probably limited the ability to observe differences in macronutrient intake (Schubert et al. 2014, in revision; (5)). Exercise and caffeine have both been examined for their hypothesized abilities to influence appetite perceptions (5, 7, 12). In agreement with most research, the exercise bout in the present study caused a transient suppression of hunger and the desire to eat while simultaneously increasing satisfaction and fullness (7), but this effect was short-lived and had dissipated within 30 min. In contrast, caffeine ingestion had no additive effect to exercise-induced alterations in appetite, despite a significant reduction in fat consumption and a moderate, although not statistically significant, decrease in EI in the EX+CAF trial compared to EX. Interestingly, changes in appetite perceptions do not appear to occur when caffeine and coffee are ingested at rest (23, 24).

The ability of caffeine to manipulate some psychological variables is well established (45). In agreement with prior research, we found caffeine attenuated RPE during exercise (16) and trended towards a reduction in muscle pain (37). We also observed a trend for improved pleasure/displeasure as assessed via the Feeling Scale (27), which is also in agreement with a prior study that utilized a similar exercise protocol in endurance-trained men (4). Caffeine also improved enjoyment of physical activity as assessed via a validated questionnaire (33). Schrader and colleagues previously reported, using Likert scales, that caffeine supplementation increased liking of physical activity in sedentary women after two weeks of caffeine paired with 30 min of moderate physical activity (40). The present study expanded these findings by showing that caffeine supplementation improved enjoyment of a moderate exercise task in recreationally active men and

women. It has been reported that both pleasure and enjoyment are associated with exercise participation and compliance; thus caffeine supplementation may be a prospective strategy to improve participation (20). Furthermore, these changes in enjoyment may be due to central effects of caffeine on adenosine and other neurotransmitters such as dopamine (22).

Exercise caused a significant perturbation in lactate levels, and this was independent of caffeine ingestion. Glucose levels were also unaffected by caffeine ingestion. Exercise led to significantly greater triglyceride levels compared to CON, and caffeine supplemented this increase. The increase in triglycerides or other markers of fat metabolism (glycerol/free-fatty acids) has been observed in response to caffeine ingestion before (1, 17), but whether increased appearance in the circulation is indicative of increased oxidation has been hotly debated. Though we observed a relationship between fat oxidation and triglycerides, correlation does not imply causation. Since this study did not use tracer methodology, the precise fate of the circulating triglycerides cannot be known.

There are a number of prospective mechanisms to support our findings that caffeine and exercise together result in significantly greater fat oxidation and energy expenditure, and a larger energy deficit when compared to exercise alone. First, as mentioned above, caffeine has well-known influences on SNS activity, and this can lead to increased ventilation, lipid shuttling and oxidation. Supporting this, a number of relationships between energy expenditure, ventilation, and fat oxidation were observed during exercise. Second, caffeine may manipulate mood by its antagonism of adenosine receptors and also increase serotonin and dopamine release (22, 35); higher dopamine levels may attenuate caloric intake or the drive to eat (11). Increased positive affect has been associated with decreases in energy intake (48), which were also observed in the present study. The ability of caffeine to influence neural networks may also have contributed to the reduction in energy intake, but this is purely speculative because to our knowledge no study has yet examined the influence of caffeine on brain areas involved in appetite and feeding behavior in response to food stimuli. Finally, caffeine has been shown to increase hippocampal brain-derived neurotropic factor (BDNF), which has significant roles in the control of energy metabolism (34, 38). Clearly, further research is required to understand the exact mechanisms underpinning the changes in EE and EI observed in the present study.

Limitations

The primary limitation of the present study is the relatively short duration of observation. We did not monitor physical activity, energy expenditure, or energy intake after the participants left

the laboratory; thus we cannot exclude the possibility that participants may have compensated for exercise or control conditions by altering their activity patterns or food intake. However, the magnitude of any potential compensatory behaviors are likely to be small given the conclusions of a recent systematic review on exercise and its impacts on non-exercise activity and energy expenditure (50), and results of earlier studies reporting that caffeine did not influence free-living energy expenditure or short bouts of voluntary physical activity (31, 32). As energy expenditure was also extrapolated from intermittent measures, it may also be overestimated.

The other significant limitation of this study is that we did not include a resting caffeine condition. However, given the conclusions of the meta-analysis by Hursel and colleagues (28) on caffeine's influence on energy expenditure and fat metabolism, in addition to our prior work on caffeine/coffee and energy intake at rest (Schubert et al., in revision), it can be inferred that a resting caffeine condition may have shown a small increase in energy expenditure but no change in energy intake. Even if caffeine did increase resting EE and fat oxidation, the reported dose-response increases of 0.44 $kJ \cdot mg^{-1}$ and 0.01 $g \cdot mg^{-1}$ reported by Hursel et al. (~180 kJ and 4.1 g for the mean caffeine dose of this study) would likely not have resulted in a significant difference from the total energy expended during the control trial; though it is possible fat oxidation may have been greater (28).

Finally, it must be noted that many individuals attain their dietary caffeine via coffee or other drinks that may have significant caloric value (i.e. lattes, colas, energy drinks) (36). Therefore, future studies may wish to examine the role of these beverages in conjunction with exercise on metabolism and energy intake from an energy balance perspective.

Future directions

Future research should consider conducting similar investigations in overweight/obese individuals, since these individuals are the ones who are most in need of an intervention to improve body composition and compliance when beginning an exercise program. Manipulating the exercise mode, duration, and intensity with caffeine ingestion may also prove useful for determining the best strategy for a negative energy balance with minimal compensation, particularly if high-intensity training is used. Recent studies have suggested that high-intensity interval training does not lead to changes in short-term energy intake and may even decrease EI at a subsequent meal; and also elicits similar 24 h energy expenditure to traditional endurance exercise (12, 42, 44). Caffeine ingestion may reduce the higher pain and perceived exertion associated with an interval training protocol, and based on our results also increase enjoyment; this could possibly improve long-term compliance.

The relationships between psychological variables related to exercise enjoyment, affect, and pleasure/displeasure also deserve further attention in an overweight/obese population – the ability to manipulate these variables in a positive manner via nutrition may prove highly useful for practitioners. Additionally, examining biomarkers of metabolism and energy regulation such as BDNF, leptin, thyroid hormones, and gastrointestinal hormones may provide mechanisms and insights into how caffeine and exercise interact to manipulate energy balance.

Conclusions

The results of this study indicate that consuming caffeine before and after a bout of strenuous exercise increased energy expenditure and fat oxidation while improving exercise enjoyment. Non-significant decreases in energy intake were also observed with caffeine. While the increases observed in energy expenditure and fat oxidation compared to a normal exercise bout were relatively small, their effects over time could be important for weight maintenance and/or weight loss. The long-term implications of caffeine supplementation during an exercise program to improve participation and manipulate energy expenditure and substrate metabolism for weight loss and energy balance merit further investigation.

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Figure legend

Figure 1 – Schematic of experimental trials

 , blood sample;  , caffeine dose (3 mg/kg BM); downward solid arrows represent visual analog scales for appetite; upward dashed arrow represents liquid breakfast at 150 min; grey boxes represent gas exchange data collection.

Figure 2 – Individual energy intake for the control (CON; open bars), exercise-placebo (EX; grey bars), and exercise-caffeine (EX+CAF; black bars) trials (A) and individual relative energy intake for control (CON; open bars), exercise-placebo (EX; grey bars), and exercise-caffeine (EX+CAF; black bars) trials (B). N = 14.

Figure 3 – Perceptual appetite responses during control (CON; ▲), exercise-placebo (EX; ○), and exercise-caffeine (EX+CAF; ●) trials for (A)hunger and (B)satisfaction. Values are means with their standard errors represented by vertical bars. Hashed rectangle represents exercise or rest; downward arrow at 150 min represents liquid breakfast and 2nd caffeine dose consumption. * P < 0.05 vs. CON. N = 14.

Figure 4 – Blood metabolite concentrations during control (CON; ▲), exercise-placebo (EX; ○), and exercise-caffeine (EX+CAF; ●) trials for (A) glucose, (B) lactate, and (C) triglycerides. N = 8. Values are means with their standard errors represented by vertical bars. Hashed rectangle represents exercise or rest; downward arrow at 150 min represents liquid breakfast and 2nd caffeine dose consumption. a, P < 0.05 EX+CAF vs. CON; b, P < 0.05 EX+CAF vs. EX; c, P < 0.05 EX vs. CON

Table 1 – Participant Demographics (Mean ± SD)

Variable	Men (n = 6)	Women (n = 8)	All (n = 14)
Age (yr)	25.7 ± 3.6	24.4 ± 5.2	24.9 ± 4.4
Height (cm)	181.1 ± 6.4*	166.2 ± 4.5	172.6 ± 9.2
Weight (kg)	80.9 ± 13.5*	59.0 ± 7.9	68.4 ± 15.1
BMI ($kg \cdot m^{-2}$)	24.5 ± 2.4*	21.3 ± 1.9	22.7 ± 2.6
Sum of skinfolds (mm)	37 ± 14	41 ± 11	40 ± 12
$\dot{V}O_2max$ ($L \cdot min^{-1}$)	3.9 ± 0.6*	2.8 ± 0.4	3.27 ± 0.74
$\dot{V}O_2max$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	48.7 ± 8.4	46.7 ± 8.5	47.6 ± 8.2
Power output at $\dot{V}O_2max$ (W)	338 ± 48*	248 ± 33	286 ± 60
Power output ($W \cdot kg^{-1}$)	4.3 ± 0.8	4.3 ± 0.7	4.25 ± 0.7
Caffeine intake ($mg \cdot d^{-1}$)	210 ± 225	202 ± 184	206 ± 194
Caffeine dosage (6 $mg \cdot kg^{-1}$)	485 ± 81*	354 ± 48	410 ± 91
TFEQ Restraint	5.7 ± 3.8	7.5 ± 3.9	6.7 ± 3.8
TFEQ Disinhibition	3.5 ± 2.9	4.3 ± 2.1	3.9 ± 2.4
TFEQ Hunger	4.0 ± 2.1	5.4 ± 1.8	4.8 ± 2.0

TFEQ, Three-Factor Eating Questionnaire

* = significantly different from women (independent samples *t*-test; $P < 0.05$)

Table 2 – Energy expenditure and substrate metabolism in the resting period, exercise period, and recovery periods (Mean ± SD)

	CON	EX	EX+CAF
Resting period (0-60 min)			
EE (kJ)	369 ± 62	361 ± 78	420 ± 99*†
CHO Ox (g)	13 ± 3	12 ± 3	13 ± 4
FAT Ox (g)	4.5 ± 1.1	4.1 ± 1.0	5.4 ± 1.4*†
RER	0.80 ± 0.03	0.81 ± 0.03	0.79 ± 0.02
Exercise/rest period (60-120 min)			
EE (kJ)	414 ± 75	3296 ± 604*	3390 ± 673*†
CHO Ox (g)	14 ± 3	146 ± 31*	138 ± 31*†
FAT Ox (g)	4.6 ± 1.6	24.3 ± 7.2*	30.4 ± 9.6*†
RER	0.82 ± 0.02	0.90 ± 0.02*	0.88 ± 0.03*†
Recovery period 1 (120-180 min)			
EE (kJ)	461 ± 89	488 ± 96	505 ± 95
CHO Ox (g)	15 ± 3	15 ± 7	13 ± 3
FAT Ox (g)	5.5 ± 1.8	6.5 ± 2*	7.4 ± 2*
RER	0.83 ± 0.02	0.82 ± 0.04	0.80 ± 0.02*
Recovery period 2 (180-240 min)			
EE (kJ)	438 ± 88	438 ± 73	517 ± 90*†
CHO Ox (g)	18 ± 5	16 ± 4	17 ± 5
FAT Ox (g)	3.6 ± 2.0	4.5 ± 1.1	6.2 ± 2.3*†
RER	0.86 ± 0.03	0.84 ± 0.02	0.82 ± 0.02*
Total trial (0-240 min)			
EE (kJ)	1706 ± 278	4582 ± 787*	4832 ± 910*†
CHO Ox (g)	60.7 ± 9.3	188.5 ± 40.1*	178.9 ± 38.5*

FAT Ox (g)	18.3 ± 5.7	37.6 ± 7.8*	48.0 ± 11.2*†

n = 14, CON, control; EX, exercise-placebo; EX+CAF, exercise-caffeine; SD, standard deviation; EE, energy expenditure; CHO Ox, carbohydrate oxidation; FAT Ox, fat oxidation

*****Mean value is significantly different from CON ($P < 0.05$)

†Mean value is significantly different from EX ($P < 0.05$)

Table 3 – Energy and macronutrient intake at the *ad libitum* test meal (Mean ± SD)

	CON	EX	EX+CAF
Energy intake (kJ)	3912 ± 1393	3911 ± 1822	3194 ± 1541
CHO Intake (g)	97 ± 36	104 ± 46	86 ± 34
FAT Intake (g)	38 ± 17	38 ± 20	30 ± 18*
PRO Intake (g)	38 ± 15	37 ± 20	33 ± 20
Weight of meal eaten (g)	551 ± 166	523 ± 178	450 ± 183*
Relative energy intake (kJ)	2206 ± 1347	-671 ± 1521*	-1638 ± 1309*†

n = 14, CON, control; EX, exercise-placebo; EX+CAF, exercise-caffeine; SD, standard deviation; CHO, carbohydrate; PRO, protein

*****Mean value is significantly different from CON ($P < 0.05$)

†Mean value is significantly different from EX ($P < 0.05$)