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Effects of exercise and thermal stress on caffeine pharmacokinetics in men and eumenorrheic women

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McLean, C., and T. E. Graham. Effects of exercise and thermal stress on caffeine pharmacokinetics in men and eumenorrheic women. *J Appl Physiol* 93: 1471–1478, 2002; 10.1152/japplphysiol.00762.2000.—The influence of gender, exercise, and thermal stress on caffeine pharmacokinetics is unclear. We hypothesized that these factors would not have an effect on the metabolism of caffeine. Eight women participated in four 8-h trials and six men participated in two 8-h trials after the ingestion of 6 mg/kg caffeine. The women performed two resting trials (1 in the follicular phase and 1 in the luteal phase of the menstrual cycle) and two exercise trials (90 min of cycling exercise at 65% of maximal O_2 uptake, 1 h after caffeine ingestion) in the follicular phase (1 without and 1 with an additional thermal stress). The men performed one exercise and one resting trial. Menstrual cycle, gender, and exercise, with or without an additional thermal stress, had no effect on the pharmacokinetic measurements or urine caffeine. There was a trend for higher plasma caffeine and lower plasma paraxanthine concentrations in the women. These results confirm that gender, exercise, and thermal stress have no effect on caffeine pharmacokinetics in men and women.

methylxanthines; dehydration; cytochrome *P*-450; gender; menstrual cycle

CAFFEINE HAS BEEN DEMONSTRATED to be a potent ergogenic aid, particularly during prolonged endurance exercise (14, 15, 32). As a result, its use in sport is monitored through urine caffeine levels, with the maximal allowable limit set at 12 μg/ml by the International Olympic Committee (IOC). Ironically, only a small percentage (1–5%) of the ingested dose is excreted in urine (18). The literature is inconsistent regarding the impact of gender, exercise, and dehydration on the absorption, distribution, metabolism, and elimination of caffeine.

Orally administered caffeine is completely absorbed from the stomach and small intestine. It is 100% bioavailable, and complete absorption occurs within 45 min. It is widely distributed in total body water (4). Caffeine is eliminated by apparent first-order kinetics that can be described by a one-compartment open-model system. There is little evidence of hepatic first-pass metabolism (10).

Caffeine is metabolized in the liver by the cytochrome *P*-450 enzyme system. Cytochrome *P*-450 1A2 (CYP1A2) is involved in the major pathway for caffeine metabolism. In human adults, this biotransformation accounts for 83.9 ± 5.4% of the primary degradation of caffeine and leads to the formation of paraxanthine. Theobromine and, to a minor extent, theophylline also accumulate in plasma after caffeine ingestion. These metabolites account for 12.2 ± 4.1 and 3.7 ± 1.3% of caffeine's degradation. Each of the primary demethylation products is further metabolized to form a variety of xanthines, uric acids, and uracils (18). The kidney is responsible for their elimination, and the metabolites appear in the urine as quickly as they are formed as a result of active renal secretion (4).

A number of lifestyle factors have been shown to have an effect on the metabolism and elimination of caffeine and, thus, may contribute to inaccurate drug testing in sport. Acute and chronic exercise (3, 4, 6, 11, 19, 23, 36, 38), smoking (35), obesity (9, 19), and dietary factors (20, 25, 36) have been reported to alter the pharmacokinetics of caffeine. Collomp et al. (11) examined caffeine kinetics over an 8-h period at rest and with exercise during the 1st h. Exercise raised the maximal plasma caffeine level and reduced the plasma half-life ($t_{½}$) and volume of distribution (V_d) yet, remarkably, did not alter the clearance of caffeine (Cl_{AUC}). Schlaeffer et al. (29) studied theophylline kinetics at rest and with two intensities of exercise (light and moderate) in a normal and hot environment: the $t_{½}$ was prolonged under all exercise conditions, and V_d was decreased by the moderate and light exercise in heat trials. Other studies that have examined the pharmacokinetics of methylxanthines have produced variable results (3, 19). Although there is evidence to suggest that methylxanthine kinetics may be affected by exercise, these studies have failed to control for factors that alter the intrinsic ability of the liver to metabolize caffeine (e.g., level of fitness, diet, menstrual cycle of female subjects, caffeine habits).

Exercise training has been associated with enhanced hepatic drug metabolism. A 70% increase in CYP1A2 activity has been reported in male subjects after 30

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days of vigorous (8–11 h/day) training (36). Boel et al. (6) reported a significant positive correlation between improvements in maximal O_2 uptake ($\dot{V}O_{2\,max}$) and the metabolism of antipyrine (a drug that undergoes a metabolism similar to caffeine). In animal studies, an increase (38) and no change (23) in hepatic microsomal enzymes or their activity have been found after endurance training.

Although in a review (4) it was stated that there are no gender differences in caffeine metabolism, oral contraceptive use (1, 26, 27) and pregnancy (2) result in a decrease in caffeine elimination. The effect of the menstrual cycle is unclear. Caffeine clearance is unchanged (40) or decreased (21) in the luteal phase of the menstrual cycle compared with the follicular phase. The few studies that have evaluated the effect of hormones on caffeine metabolism suffer from methodological problems. These studies have included women of wide reproductive age (e.g., 18–35 yr) (27), have not controlled for the reproductive (or hormonal) status of the subjects, and have not measured any of the reproductive hormones to document menstrual cycle position.

Therefore, the following investigation was designed to evaluate *1*) the effect of the menstrual cycle, exercise, and thermal stress on the pharmacokinetics of caffeine in trained women and *2*) the effect of gender and an acute bout of exercise on the metabolism of caffeine and urine caffeine levels in men and women. It was hypothesized that gender and exercise, with and without an additional thermal stress, would not alter caffeine pharmacokinetics and urine caffeine levels when menstrual status and lifestyle factors were controlled.

METHODS

Eight active, eumenorrheic women and six active men volunteered to participate in the study. Before inclusion in the study, each subject was required to answer a questionnaire detailing their medical and physical activity history, drug use, and caffeine intake from food, beverage, and drug sources. Female subjects were questioned regarding their menstrual status, oral contraceptive use, and age at menarche. All subjects were healthy, none were smokers or used drugs (including oral contraceptive steroids), and all were regularly involved in endurance activities (>3 times/wk). The average daily caffeine consumption for both groups was 68.9 mg (range 25–225 mg/day). All female subjects reported regularly occurring menstrual cycles (24–35 days) over the previous 1 yr. The subjects were matched for age, caffeine habits, and level of fitness (Table 1). The study was explained verbally and in written form to each subject, and a signed consent form was received. The University of Guelph Ethics Committee approved the research protocol.

Pretesting Protocol

Each subject reported to the laboratory before testing for an incremental $\dot{V}O_{2\,max}$ test on a cycle ergometer. After a 3-min warm-up, the workload was increased every 2 min until the subject was unable to maintain the required pedal frequency for >15 s or until volitional exhaustion was reached. The subjects were verbally encouraged by the investigators. Respiratory parameters were determined at each exercise intensity. The subjects were matched for fitness level ($\dot{V}O_{2\,max}$): 50.6 ± 1.20 and 50.7 ± 1.7 $ml \cdot kg^{-1} \cdot min^{-1}$ for women and men, respectively. From the $\dot{V}O_{2\,max}$ test, a power output equivalent to 60–65% $\dot{V}O_{2\,max}$ was determined. On a separate occasion, each subject reported to the laboratory for a practice cycle test to ensure that the predicted power output elicited the desired O_2 consumption and to familiarize the subject with the exercise protocol.

Before testing, body composition was determined via hydrostatic weighing. Body density and percent body fat were calculated on the basis of the Siri equation (30). Although not matched for body composition, the subjects were similar in their percent body fat (21.0 ± 0.7 and 16.4 ± 1.8% for women and men, respectively) and absolute fat mass (12.1 ± 0.64 and 13.0 ± 1.67 kg for women and men, respectively).

The women were required to document their menstrual cycle (the 1st day of menstruation and the number of days until the beginning of a subsequent menstrual cycle) for a minimum of two cycles before testing. On the basis of this information, each woman's average cycle length was calculated. The 1st day of menstruation was designated *day 1*. The women performed four experimental trials: three in the follicular phase of their menstrual cycle (the day corresponding to one-fourth of their average cycle length) and one during the luteal phase of their menstrual cycle (the day corresponding to three-fourths of their average cycle length). On average, the follicular testing was performed on *day 7* ± 1 day, and the luteal testing was performed on *day 21* ± 1 day. Menstrual cycle phase was confirmed by hormonal measurements (see below).

Experimental Protocol

During the 3 days before testing, all subjects were required to complete diet records. They were asked to abstain from alcohol, all caffeine-containing substances, cruciferous vegetables, and charcoal-broiled meat for 48 h before testing. The subjects were instructed to avoid heavy exercise for 24 h before testing. Before arriving at the laboratory, the subjects were asked to consume a light meal. The subjects were instructed to follow the same pretesting procedures and maintain a similar diet before each testing session.

The women performed four randomized 8-h trials. They performed two resting trials [1 in the follicular phase (FR) and 1 in the luteal phase (LR)] and two exercise trials in the follicular phase [1 with an extra heat stress (FEH) and 1 without (FE)]. Thus testing was conducted for three consecutive menstrual cycles. On the basis of the results of the

Table 1. *Subject characteristics*

	n	Age, yr	Weight, kg	Body Fat, %	Absolute Body Fat, kg	$\dot{V}O_{2max}$, $ml \cdot kg^{-1} \cdot min^{-1}$	Caffeine Ingestion, mg/day	Menstrual Cycle Length, days	Age at Menarche, yr
Female	8	22.8 ± 0.73	57.4 ± 2.10	21.0 ± 0.73	12.1 ± 0.64	50.7 ± 1.65	58.3 ± 33.3	28.5 ± 0.96	13.9 ± 0.55
Male	6	25.8 ± 1.72	78.0 ± 4.00*	16.4 ± 1.77*	13.0 ± 1.67	50.6 ± 1.23	76.9 ± 18.8		

Values are means ± SE; *n*, no. of subjects. $\dot{V}O_{2max}$, maximal O_2 uptake. Caffeine ingestion was estimated on the basis of data from Somani and Gupta (31). Menstrual cycle length was determined from the average of the 2 mo before testing. *Significant gender difference ($P < 0.05$).

women, the extra heat stress trial was not included in the experiments with men. As a result, the men performed two randomized 8-h trials [1 resting trial (MR) and 1 exercise trial (ME)].

On arrival at the laboratory, the subjects were asked to void their bladder. A catheter was placed in an antecubital vein and was kept patent with a normal saline drip that included 0.1% heparin. After a resting blood sample (10 ml) was obtained, the subjects ingested 100 ml of water with 6 mg/kg caffeine in capsule form [a dose of 3–9 mg/kg has been shown to be ergogenic (14, 15, 31), and 6 mg/kg has been shown to saturate the cytochrome *P*-450 enzyme system (15)]. This dose is approximately equal to the caffeine content of two large mugs of coffee (31). Blood samples were taken every 60 min for 4 h and again at 8 h after caffeine ingestion (0, 1, 2, 3, 4, and 8 h). A total of six blood samples were taken (45 ml).

During the resting trials, the subjects remained seated at rest for the duration of the experiment. After caffeine ingestion in the exercise trials, the subjects were weighed in their regular street clothes and then asked to change into their clothes for exercise. In ME and FE, the subjects exercised wearing shorts, T-shirt, and their underclothes. In FEH and FE, rectal and skin temperatures were monitored. In these trials, the subjects were required to insert a rectal temperature probe 10–12 cm and attach thermistors to sites on the abdomen and chest. To induce an extra thermal stress in FEH, the subjects wore a long-sleeved shirt, sweatshirt, and sweatpants in addition to the clothes worn in FE. The extra clothing was provided to the subjects and was uniform in nature. The laboratory was kept at a constant temperature of 21°C.

At 1 h after caffeine ingestion in the exercise trials, the subjects exercised for 1.5 h on a cycle ergometer at 65% $\dot{V}O_{2\,max}$. During the exercise, expired air was analyzed every 10 min for the first 40 min and at 1 h of exercise. Heart rates were recorded every 15 min throughout the exercise. After exercise, the subjects towel dried and were reweighed in their street clothes. Body weight loss due to sweating was calculated on the basis of the change in body weight before and after exercise. A postexercise urine sample was obtained within 30 min of completion of exercise. During MR, FR, and LR, a urine sample was obtained at the time corresponding to the postexercise sample in ME, FE, and FEH.

All beverages and food were supplied during the experimental sessions. The subjects were allowed to ingest 20 ml of water during exercise. At 3 h after caffeine ingestion in all trials, the subjects consumed 300 ml of juice. After the 4-h blood sample was obtained, the subjects ingested a standard lunch with 300 ml of water. At 6 h after caffeine ingestion, the subjects consumed another 300 ml of juice.

Analyses

Expired gas samples were analyzed for fraction of O_2 and CO_2 with an O_2 analyzer (model S-3A, Applied Electochemical) and a CO_2 analyzer (model LB-2, SensorMedics, Oakville, ON, Canada). Expired volume was determined with a Parkinson-Cowan volume meter. The analyzers were calibrated with gases of known concentrations. The volume meter was calibrated with a calibrated syringe.

Blood samples were immediately separated into two aliquots. For the first blood sample from the women, an aliquot of 3 ml was transferred to a nonheparinized tube for estradiol analysis. A 6-ml aliquot was transferred to a sodium heparinized tube for methylxanthine analysis. For the first blood sample, this tube also included an extra 3 ml of blood for progesterone analysis. Hematocrit was measured by high-speed centrifugation from the heparinized tube of each blood sample.

The urine pH was adjusted (4 N HCl) to 3.5 for caffeine analysis. All blood and urine samples were stored at −80°C for later analysis.

Plasma methylxanthines and urine caffeine were measured using fully automated HPLC (Waters). Briefly, 150 μl of plasma were added to ~40 mg of ammonium sulfate and 50 μl of 0.05% acetic acid. After 25 μl of internal standard solution (7-β-hydroxypropyl theophylline) and 3 ml of chloroform-isopropyl alcohol (85:15, vol/vol) extracting solvent were added, the mixture was vortexed for 30 s and centrifuged for 10 min at 2,500 rpm. The organic phase was transferred and dried under O_2-free N_2 and resuspended in HPLC mobile-phase solvent (3% isopropanol, 0.05% acetic acid, and 0.5% acetonitrile), and 100 μl were injected onto a 5-μm C_{18} column (IP, Ultrasphere, Beckman). Methylxanthines were measured at 282-nm wavelength. Reagents for standards were obtained from Sigma-Aldrich Canada (Oakville, ON, Canada). The urine caffeine was measured with the same technique; however, the sample volume was increased to 200 μl, and 40 μl of internal standard were used.

Six independent samples were freshly extracted and injected, in duplicate, on 3 independent days to validate the HPLC method. The duplicate analysis for each sample on a given day was averaged, and then the value for each sample for the 3 independent days was averaged. The mean methylxanthine concentrations of these samples are presented in Table 2.

Serum estradiol and plasma progesterone were measured with a ^{125}I radioimmnunoassay kit (Coat-a-Count Estradiol and Progesterone Kit, Diagnostic Products, Los Angeles, CA).

Calculations

The peak caffeine concentration for each trial was determined from the plasma caffeine data, and the time to reach peak caffeine concentration corresponded with this. The calculation of kinetic parameters for caffeine was based on first-order kinetics and a one-compartment model (32). The plasma caffeine data were fit to an equation ($y = A * e^{-kt}$) where t is time, A is the peak caffeine concentration, y is the final caffeine concentration (8-h sample), k is constant of elimination (identified as K_{el} within the manuscript) from the peak value to the final sample using a computer program (Fig P Software, 1991). The elimination constant (K_{el}) was determined from the slope of the line of best fit. Area under the concentration-time curve (AUC) was determined by the trapezoidal rule from *time 0* to infinity (28). Cl^{AUC} was calculated as follows

Table 2. *Methylxanthine concentrations for independent samples*

Sample	Methylxanthine Concentration, μM			
	Theobromine	Paraxanthine	Theophylline	Caffeine
A	0.64 ± 0.01	5.24 ± 0.12	0.53 ± 0.02	37.4 ± 0.89
B	0.70 ± 0.01	5.58 ± 0.06	0.45 ± 0.02	31.7 ± 1.05
C	0.64 ± 0.00	5.33 ± 0.07	0.45 ± 0.00	32.4 ± 1.74
D	0.83 ± 0.01	5.50 ± 0.07	0.55 ± 0.01	30.1 ± 0.75
E	2.61 ± 0.02	3.49 ± 0.00	0.50 ± 0.01	43.4 ± 0.03
F	1.00 ± 0.02	7.40 ± 0.19	0.63 ± 0.00	33.6 ± 0.59

Values are means ± SE. Samples were freshly extracted and analyzed on 3 independent days to assess validity of HPLC method.

$$Cl^{AUC} = F * dose/AUC * BW\ (ml \cdot min^{-1} \cdot kg^{-1})$$

where BW is body weight (kg) and F is bioavailable fraction equal to 1 (10). V_d was calculated as follows

$$V_d = Cl^{AUC}/K_{el}\ (l/kg)$$

and $t_{1/2}$ was calculated as follows

$$t_{1/2} = 0.693/K_{el}\ (h)$$

(16, 28).

Statistics

The effect of menstrual cycle (FR vs. LR) on the pharmacokinetic measurements and urine caffeine was evaluated with a paired *t*-test. A two-way repeated-measures ANOVA was used to analyze the effect of menstrual cycle position on methylxanthine concentrations. There was no difference between FR and LR in methylxanthine concentrations, pharmacokinetic measurements, and urine caffeine (Tables 3 and 4). As a result, FR and LR were averaged to produce a combined, resting trial (FRC) for the women. The effect of exercise with an additional thermal stress (FE vs. FEH) on the pharmacokinetic measurements and urine caffeine was evaluated with a paired *t*-test. Methylxanthine concentrations, pharmacokinetic measurements, and urine caffeine were analyzed with a two-way repeated-measures ANOVA. The effect of gender and exercise on the pharmacokinetic measurements and urine caffeine was evaluated with an one-way repeated-measures ANOVA. Subject characteristics were compared with a *t*-test. A protected least significant difference post hoc test was used to identify significant differences. $P < 0.05$ was considered significant.

Table 3. *Methylxanthine concentrations during FR, LR, FE, and FEH*

Trial	Time, h	Methylxanthine Concentration, μM			
		Theobromine	Paraxanthine	Theophylline	Caffeine
FR	0	0.27 ± 0.13	0.44 ± 0.33	0.03 ± 0.03	0.29 ± 0.27
	1	0.68 ± 0.16	3.28 ± 0.57	0.32 ± 0.07	41.1 ± 2.87
	2	1.07 ± 0.30	4.91 ± 0.79	0.48 ± 0.05	43.5 ± 2.16
	3	1.41 ± 0.34	6.87 ± 0.73	0.74 ± 0.08	39.3 ± 2.65
	4	1.60 ± 0.29	8.23 ± 0.67	0.91 ± 0.08	35.6 ± 2.51
	8	2.00 ± 0.20	10.1 ± 0.69	1.39 ± 0.11	24.7 ± 2.80
LR	0	0.34 ± 0.14	0.21 ± 0.21	0.05 ± 0.05	0.29 ± 0.29
	1	0.65 ± 0.12	3.47 ± 0.56	0.27 ± 0.05	48.9 ± 4.90
	2	1.07 ± 0.16	5.98 ± 0.67	0.61 ± 0.10	50.2 ± 2.73
	3	1.33 ± 0.13	7.64 ± 0.77	0.78 ± 0.09	43.3 ± 1.28
	4	1.55 ± 0.16	8.66 ± 0.60	0.94 ± 0.10	40.9 ± 2.24
	8	2.18 ± 0.12	11.4 ± 0.61	1.39 ± 0.10	26.9 ± 2.68
FE	0	0.16 ± 0.04	0.27 ± 0.10	0.06 ± 0.04	0.05 ± 0.03
	1*	0.45 ± 0.08	2.77 ± 0.40	0.28 ± 0.06	39.1 ± 4.42
	2	0.88 ± 0.14	4.89 ± 0.55	0.52 ± 0.05	43.4 ± 2.16
	3	1.11 ± 0.14	6.30 ± 0.77	0.68 ± 0.07	41.2 ± 2.42
	4	1.36 ± 0.12	7.84 ± 0.89	0.87 ± 0.09	33.8 ± 3.06
	8	2.08 ± 0.15	10.5 ± 0.81	1.24 ± 0.11	24.5 ± 2.44
FEH	0	0.24 ± 0.06	0.17 ± 0.10	0.03 ± 0.02	0.09 ± 0.08
	1*	0.70 ± 0.08	2.99 ± 0.29	0.26 ± 0.04	34.3 ± 3.00
	2	0.92 ± 0.09	4.70 ± 0.36	0.41 ± 0.04	40.7 ± 3.11
	3	1.21 ± 0.10	5.80 ± 0.54	0.59 ± 0.05	38.1 ± 2.17
	4	1.43 ± 0.07	7.16 ± 0.74	0.69 ± 0.07	35.9 ± 1.95
	8	1.97 ± 0.11	10.5 ± 0.79	1.10 ± 0.10	26.4 ± 2.98

Values are means ± SE. FR, follicular rest; LR, luteal rest; FE, follicular exercise; FEH, follicular exercise with an extra heat stress. Two subjects were dropped from LR, because their hormonal data did not confirm ovulation. *Start of exercise (total duration = 1.5 h).

RESULTS

Subject Characteristics

The men and women did not differ in their age, $\dot{V}O_{2\ max}$, or estimated daily caffeine ingestion. The men were significantly heavier and had lower percent body fat than the women ($P < 0.05$), yet they had almost identical absolute body fat (Table 1).

Trials With Women

Hematocrit, estradiol, and progesterone. There was no difference between any of the trials in hematocrit measurements. Furthermore, there was no significant difference between the three follicular trials in steroid hormones. Mean progesterone and estradiol concentrations in FR (2.12 ± 0.67 and 0.14 ± 0.03 nM, respectively), FE (1.48 ± 0.33 and 0.13 ± 0.02 nM, respectively), and FEH (1.33 ± 0.22 and 0.16 ± 0.03 nM, respectively) were within the expected ranges for the follicular phase of a normal menstrual cycle (0.48–4.5 and 0.03–0.28 nM, respectively) (12). With the exception of two subjects in LR, mean progesterone and estradiol (36.0 ± 5.3 and 0.39 ± 0.05 nM, respectively) were within the expected ranges for the luteal phase of a normal menstrual cycle (8.0–89 and 0.22–0.95 nM, respectively) (12). The two subjects were excluded from the initial analysis involving the comparison of FR and LR, inasmuch as their hormonal data did not confirm ovulation. Although there was a trend for higher plasma caffeine, there were no differences between trials in methylxanthine concentrations (Table 3) or pharmacokinetic measurements (Table 4). Because no differences were found between FR and LR, the two subjects excluded from the initial analysis of FR and LR were included when the trials were combined (FRC).

Exercise trials. There was no difference in heart rate (83 ± 5 and 84 ± 5 beats/min for FE and FEH, respectively), rectal temperature (37.3 ± 0.06 and 37.3 ± 0.04°C for FE and FEH, respectively), and abdominal (33.7 ± 0.38 and 34.5 ± 0.29°C for FE and FEH, respectively) and chest (33.6 ± 0.46 and 34.5 ± 0.30°C for FE and FEH, respectively) skin temperatures before the beginning of exercise. There was no difference between FE and FEH in average O_2 consumption during exercise (33.0 and 34.0 $ml \cdot kg^{-1} \cdot min^{-1}$, respectively). Subjects exercised at ~65% $\dot{V}O_{2\ max}$ in FE and at 66% $\dot{V}O_{2\ max}$ in FEH. However, FEH resulted in a significantly greater ($P < 0.05$) heart rate (177 ± 5.27 vs. 167 ± 7.40 beats/min), weight loss (1.51 ± 0.09 vs. 1.25 ± 0.13 kg), and chest (36.2 ± 0.22 vs. 31.3 ± 0.74°C) and abdominal (36.5 ± 0.41 vs. 32.6 ± 0.84°C) skin temperatures at the end of the 1.5-h exercise bout. Although not significant ($P = 0.10$), rectal temperatures tended to be higher in FEH than in FE (39.4 ± 0.22 vs. 38.6 ± 0.11°C). Although the thermal stress was greater in FEH, this had no effect on any of the pharmacokinetic measurements, urine caffeine, or methylxanthine concentrations (Tables 3 and 4).

Table 4. *Summary of pharmacokinetic measurements for FR, LR, FE, and FEH*

Trial	AUC, mg·h·l^{-1}	Peak Plasma [Caffeine], μM	Time to Peak [Caffeine], h	K_{el}, h^{-1}	Plasma $t_{½}$, h	V_d, l/kg	Cl_{AUC}, ml·min^{-1}·kg^{-1}
FR	116.1 ± 11.6	48.0 ± 1.43	1.75 ± 0.41	0.11 ± 0.01	7.22 ± 0.71	0.56 ± 0.05	0.93 ± 0.10
LR	117.4 ± 13.4	49.4 ± 2.80	1.60 ± 0.34	0.11 ± 0.02	7.06 ± 0.90	0.66 ± 0.08	1.12 ± 0.15
FE	99.9 ± 11.1	47.0 ± 1.80	2.06 ± 0.26	0.12 ± 0.02	6.92 ± 0.85	0.60 ± 0.03	1.14 ± 0.19
FEH	116.0 ± 15.7	43.7 ± 2.00	1.88 ± 0.23	0.10 ± 0.01	8.27 ± 1.15	0.64 ± 0.03	1.05 ± 0.18

Values are means ± SE. AUC, area under the concentration-time curve; [caffeine], caffeine concentration; K_{el}, constant of elimination; $t_{1/2}$, half-life; V_d, volume of distribution; Cl_{AUC}, plasma clearance.

Comparison of Men and Women

There was no gender difference between exercise trials in terms of O_2 consumption during exercise or weight loss due to sweating. The subjects exercised at 65% $\dot{V}_{O_2\,max}$ (33.0 and 33.4 ml·kg^{-1}·min^{-1} for women and men, respectively). Relative and absolute weight loss due to sweating was not different between women (−2.6% or 1.52 ± 0.13 kg body wt) and men (−2.3% or 1.82 ± 0.29 kg body wt).

Methylxanthines. The mean plasma theobromine, paraxanthine, theophylline, and caffeine concentrations during FRC, FE, MR, and ME are presented in Table 5. All subjects reported to the laboratory with plasma caffeine <2 μM and low dimethylxanthine concentrations. These measurements confirmed that all subjects avoided caffeine- and methylxanthine-containing foods before testing. All metabolites increased in concentration while plasma caffeine decreased over time ($P < 0.05$). Plasma caffeine was higher ($P < 0.05$) in FRC than in MR and tended to be higher in FE than in ME. Paraxanthine concentrations were lower ($P < 0.05$) in FE than in ME at 2, 3, 4, and 8 h, and a similar tendency was observed for FRC vs. MR.

Urine. There was no gender difference in urine caffeine at rest (9.18 ± 0.76 and 7.66 ± 0.81 μg/ml in MR and FRC, respectively) and during exercise (7.12 ± 0.61 and 6.10 ± 0.56 μg/ml in ME and FE, respectively). Furthermore, there was no effect of exercise on urine caffeine levels. Urine caffeine ranged from 3.57 to 10.0 μg/ml in FRC, from 4.29 to 8.43 μg/ml in FE, from 5.34 to 10.2 μg/ml in MR, and from 4.95 to 9.16 μg/ml in ME. A two- to threefold variation in urine caffeine was noted between subjects. No subjects achieved a urine caffeine level greater than the IOC limit of 12 μg/ml.

Pharmacokinetics. The pharmacokinetic measurements for FRC, FE, MR, and ME are presented in Table 6. Although there was a tendency for higher caffeine in the women, there was no difference between trials in AUC, peak, or time to peak caffeine concentration, K_{el}, Cl^{AUC}, V_d, or $t_{½}$.

DISCUSSION

The purpose of the present study was to determine the impact of exercise and an additional thermal stress on caffeine pharmacokinetics in men and women matched for caffeine habits and level of fitness. The results demonstrate that there was no effect of menstrual cycle, gender, or exercise, with or without an additional thermal stress, on the absorption, distribution, metabolism, or elimination of caffeine in matched women and men. This is in agreement with a recent study (40) that found no effect of menstrual cycle position on CYP1A2 activity (assessed by caffeine clearance after a 150-mg dose). It is, however, in disagreement with a previous report that the rise in estradiol and progesterone during the luteal phase of the normal menstrual cycle is significantly correlated with a reduction in the clearance of caffeine (21). The authors suggested that elevated estradiol concentrations inhibit caffeine metabolism as a result of substrate competition or the impairment of CYP1A2 activity. However, in vitro studies have documented that the hormone concentrations required for enzyme inhibition are higher than those achieved by oral contracep-

Table 5. *Methylxanthine concentrations during FRC, FE, MR, and ME*

Trial	Time, h	Methylxanthine Concentration, μM			
		Theobromine	Paraxanthine	Theophylline	Caffeine
		Women			
FRC	0	0.35 ± 0.12	0.37 ± 0.20	0.07 ± 0.04	0.36 ± 0.23†
	1	0.71 ± 0.13	3.22 ± 0.48	0.32 ± 0.05	42.9 ± 2.49†
	2	1.07 ± 0.20	5.17 ± 0.67	0.54 ± 0.07	43.8 ± 1.74†
	3	1.38 ± 0.19	7.18 ± 0.62	0.78 ± 0.09	40.4 ± 2.31†
	4	1.58 ± 0.18	8.47 ± 0.53	0.96 ± 0.09	37.1 ± 2.67†
	8	2.09 ± 0.13	10.8 ± 0.45	1.42 ± 0.10	25.3 ± 2.20†
FE	0	0.16 ± 0.04	0.27 ± 0.10	0.06 ± 0.04	0.05 ± 0.03
	1‡	0.45 ± 0.08	2.77 ± 0.40	0.28 ± 0.06	39.1 ± 4.42
	2	0.88 ± 0.14	4.89 ± 0.55*	0.52 ± 0.05	43.4 ± 2.16
	3	1.11 ± 0.14	6.30 ± 0.77*	0.68 ± 0.07	41.2 ± 2.42
	4	1.36 ± 0.12	7.84 ± 0.89*	0.87 ± 0.09	33.8 ± 3.06
	8	2.08 ± 0.15	10.5 ± 0.81*	1.24 ± 0.11	24.5 ± 2.44
		Men			
MR	0	1.01 ± 0.58	0	0	0
	1	1.26 ± 0.53	2.94 ± 0.39	0.23 ± 0.05	36.3 ± 3.20
	2	1.50 ± 0.43	6.02 ± 0.46	0.58 ± 0.04	38.3 ± 1.85
	3	1.85 ± 0.46	8.48 ± 0.73	0.81 ± 0.04	33.5 ± 1.45
	4	2.03 ± 0.49	8.96 ± 0.77	0.91 ± 0.09	29.7 ± 2.04
	8	2.78 ± 0.35	12.7 ± 0.89	1.26 ± 0.08	18.1 ± 1.91
ME	0	0.54 ± 0.32	0.28 ± 0.28	0.05 ± 0.05	0.09 ± 0.08
	1‡	0.69 ± 0.27	3.37 ± 0.57	0.23 ± 0.08	27.8 ± 5.36
	2	1.33 ± 0.26	6.52 ± 0.34	0.59 ± 0.03	34.2 ± 2.19
	3	1.57 ± 0.23	8.10 ± 0.64	0.77 ± 0.06	33.0 ± 2.86
	4	1.88 ± 0.27	10.5 ± 0.70	0.94 ± 0.08	28.8 ± 1.91
	8	2.50 ± 0.20	13.0 ± 0.76	1.37 ± 0.07	18.8 ± 1.93

Values are means ± SE. FRC, follicular rest (combined). *Within a treatment, significantly different time points between genders ($P < 0.05$). †Differences between genders within a treatment ($P < 0.05$). ‡Start of exercise (total duration = 1.5 h).

Table 6. *Summary of pharmacokinetic measurements for FRC, FE, MR, and ME*

Trial	AUC, mg·h·l^{-1}	Peak Plasma [Caffeine], μM	Time to Peak [Caffeine], h	K_{el}, h^{-1}	Plasma $t_{1/2}$, h	V_d, l/kg	Cl_{AUC}, ml·min^{-1}·kg^{-1}
				Women			
FRC	114.5 ± 11.3	49.8 ± 2.21	1.68 ± 0.25	0.11 ± 0.01	7.19 ± 0.69	0.67 ± 0.05	1.09 ± 0.13
FE	99.9 ± 11.1	47.0 ± 1.80	2.06 ± 0.26	0.12 ± 0.02	6.92 ± 0.85	0.60 ± 0.03	1.14 ± 0.19
				Men			
MR	81.5 ± 9.8	40.3 ± 2.90	1.83 ± 0.17	0.13 ± 0.02	5.70 ± 0.75	0.61 ± 0.05	1.30 ± 0.12
ME	82.3 ± 8.9	41.5 ± 3.06	1.92 ± 0.20	0.11 ± 0.01	6.28 ± 0.55	0.67 ± 0.04	1.27 ± 0.11

Values are means ± SE.

tive use and menstrual cycle fluctuations (22). Additionally, closer examination of the data reported by Lane et al. (21) suggests that two subjects' individual responses may have skewed the results. The majority of subjects (8 of 10) demonstrated a <4% change in caffeine clearance between the follicular and luteal phase of the menstrual cycle; however, two subjects had a dramatic (26 and 45%) reduction in caffeine clearance during the luteal phase: 1.74 and 1.29 ml·min^{-1}·kg^{-1} in follicular and luteal phases, respectively (*subject A*) and 0.72 and 0.41 ml·min^{-1}·kg^{-1} in follicular and luteal phases, respectively (*subject B*). Therefore, study size, individual differences, and differences in caffeine dose [250 mg (21) vs. 150 mg (40) vs. 6 mg/kg in the present study] may account for the discrepancy in the literature. Although our rigorous controls should have reduced individual variability, our limited sample size does increase the risk of a type IIA error and precludes the application of the results to a general population. Nevertheless, the present results are in agreement with research that has failed to note a gender difference in caffeine kinetics (8) or CYP1A2 activity once oral contraceptive use (thus, hormonal status) was controlled (8, 17, 18).

The K_{el}, V_d, and Cl_{AUC} measurements in the present study were similar to those previously reported at rest using similar dosages (7, 10, 24). There was no difference in caffeine metabolism and elimination between the resting and exercise trials. This is in agreement with the pharmacokinetic literature that has demonstrated no effect of exercise on the metabolism and elimination of drugs, such as caffeine, with a large V_d and a low hepatic extraction ratio (13, 34, 39). However, this is in contrast to previous studies of methylxanthine pharmacokinetics (11, 29).

Collomp et al. (11) examined caffeine kinetics in a group of men and women over an 8-h period at rest and with mild exercise (30% $\dot{V}O_{2\ max}$) during the 1st h. They noted that exercise increased plasma caffeine and decreased V_d and $t_{½}$, yet it had no effect on Cl_{AUC}. The authors suggested that the increase in plasma caffeine and decrease in V_d were linked to cardiocirculatory adjustments with exercise, yet there were no measurements that could confirm this hypothesis. Because Cl_{AUC} is calculated as the product of K_{el} and V_d, in this study, K_{el} must have increased to an extent similar to the decrease in V_d in response to exercise (e.g., 40–50%). As a result, the average K_{el} would be ~0.18 (value calculated on the basis of the author's published mean V_d and Cl_{AUC} data) and would be far greater than previously documented.

In contrast, a decrease in K_{el}, Cl_{AUC}, and V_d, yet an increase in $t_{½}$ of theophylline, was demonstrated with mild exercise in a heat-controlled room (40°C) (29). The authors suggested that the decrease in V_d with exercise and heat was evidence of dehydration; however, fluid loss (dehydration) was not measured. In the present study, measurements of thermal stress (e.g., weight loss due to sweating, heart rate, and body temperature) confirmed a greater thermal stress in FEH than in FE. The exercise duration in the present study was similar, yet the intensity was greater than in the previously mentioned studies (11, 29). Therefore, the degree of dehydration in this study (1.25, 1.50, and 1.82 liters for FE, FEH, and ME, respectively) should be more extreme than during the aforementioned studies. Nevertheless, there was no difference in V_d or any of the other kinetic measurements. Because caffeine is a widely distributed drug, fluid losses in this range represent <3% body weight and only ~4% of the apparent V_d. Even extreme fluid losses equivalent to 6–10% could not account for the large reductions in V_d reported by Collomp et al. (11) or Schlaeffer et al. (29). Only drugs that have a higher molecular weight than caffeine and are confined to the plasma compartment of the body may be drastically influenced by a small reduction in V_d (37).

In contrast to the above-mentioned investigations (11, 29), we found that K_{el} was not different between men and women and was not affected by exercise with or without heat. K_{el} denotes all processes, hepatic and otherwise, leading to the clearance of a drug. It is largely a function of hepatic blood flow and hepatic extraction. Alterations in hepatic blood flow should have no effect on the elimination of drugs, such as caffeine, with enzyme-limited metabolism (37). However, the hepatic extraction of these drugs can be drastically altered by factors that alter hepatic enzyme activity and/or content. Unlike the present study, subjects in the previously mentioned studies (11, 29) were not matched with respect to physical fitness. Additionally, cigarette smoking, chronic caffeine consumption, and the dietary consumption of compounds known to alter caffeine metabolism were not controlled. Furthermore, menstrual status was not considered. The women were 23–52 yr old and, therefore, could have

ranged in menstrual functioning from amenorrheic to menopausal. In the present study, hormonal measurements confirmed that two of eight women failed to ovulate. These findings highlight the importance of sex steroid measurements in establishing the menstrual status of female subjects. It is difficult to interpret the results of other studies (11, 29), since the results are contradictory and internally inconsistent.

The present study noted statistically higher caffeine concentration in FRC and a similar trend in FE compared with the male trials. Consistent with these data, paraxanthine concentrations were statistically lower in FE, and the same trend existed in FRC. Higher caffeine and lower paraxanthine concentrations in women suggest less CYP1A2 activity and may indicate that the cytochrome *P*-450 enzyme system saturates earlier in women than in men. Nonetheless, there were no gender differences in any of the kinetic measurements, and no previous literature has documented gender differences in the saturation level of CYP1A2. Further studies using different doses of caffeine would be necessary to better understand any gender differences in CYP1A2 activity.

Neither gender nor exercise with or without an additional heat stress had an effect on urine caffeine. Furthermore, no subjects achieved a urine caffeine level greater than the IOC limit. These results are in agreement with previous investigations that have evaluated urine caffeine after similar caffeine doses (15, 35).

Gender, menstrual cycle, and exercise-induced dehydration had no effect on the urine caffeine levels. The present findings have a practical application to drug testing in sport. Caffeine's use in sport is monitored through urine testing, with the upper acceptable level set at 12 μg/ml by the IOC. No subject in the present study achieved a urine caffeine concentration greater than this limit, although the ingested dose has been shown to be ergogenic (14, 15, 32). Although there was little variability in the plasma caffeine concentrations, there was wide inter- and intrasubject variability in urinary caffeine concentrations. A two- to threefold variation in urine caffeine concentrations was noted between subjects in the present study, even though all subjects received the same relative caffeine dose on the basis of their body weight. Interindividual variability in resting subject's caffeine urine concentrations on the order of 3- to 15-fold has been previously reported (5).

From the present data, it is concluded that menstrual cycle, gender, exercise, and thermal stress have no effect on the absorption, distribution, metabolism, and elimination when reproductive status and environmental and dietary factors were controlled. Gender and exercise, with or without an additional thermal stress, had no effect on urine caffeine levels, and no subjects in the present study achieved a urine caffeine level greater than the IOC limit of 12 μg/ml.

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