

ARTICLE

Effects of Common *CYP1A2* Genotypes and Other Key Factors on Intraindividual Variation in the Caffeine Metabolic Ratio: An Exploratory Analysis

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The caffeine metabolic ratio is an established marker for cytochrome P450 (CYP) 1A2 activity. Optimal sample size calculation for clinical pharmacokinetic xenobiotic–caffeine interaction studies requires robust estimates of interindividual and intraindividual variation in this ratio. Compared with interindividual variation, factors contributing to intraindividual variation are less defined. An exploratory analysis involving healthy nonsmoking non-naïve caffeine drinkers (1–3 cups/day; 12 men, 12 women) administered caffeine (160 mg) on five occasions evaluated the effects of *CYP1A2* induction status (based on genotype) and other factors on intraindividual variation in *CYP1A2* activity. Results were compared with those from previous studies. Regardless of whether a hyperinducer (*CYP1A2*1A/*1F* or *CYP1A2*1F/*1F*) or normal metabolizer (*CYP1A2*1A/*1A*, *CYP1A2*1C/*1F*, or *CYP1A2*1C*1F/*1C*1F*), sex, age, oral contraceptive use by women, and smoking status, intraindividual variation was $\leq 30\%$. A value of 30% is proposed for optimal design of pharmacokinetic xenobiotic–caffeine interaction studies. Prospective studies are needed for confirmation.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Optimal sample size calculation for cytochrome P450 (CYP)–mediated pharmacokinetic xenobiotic–drug interaction studies requires robust estimates of both interindividual and intraindividual variation in enzyme activity. Relative to interindividual variation in *CYP1A2* activity, commonly assessed by the caffeine metabolic ratio, factors contributing to intraindividual variation are less defined.

WHAT QUESTION DID THIS STUDY ADDRESS?

An exploratory analysis involving a well-characterized cohort of 24 nonsmoking volunteers administered caffeine on five separate occasions was conducted to investigate the effects of *CYP1A2* induction status (based on genotype) and other key factors on the intraindividual variation in *CYP1A2* activity.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

Intraindividual variation in the caffeine metabolic ratio, as assessed by the coefficient of variation, was generally $\leq 30\%$, regardless of *CYP1A2* induction status, sex, and oral contraceptive use by women.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

An intraindividual variation of 30% is proposed for calculating the optimal sample size needed to detect a change in the caffeine metabolic ratio for *CYP1A2*–mediated xenobiotic–drug interaction studies. Prospective studies are needed to confirm this observation.

Cytochrome P450 (CYP) 1A2 is considered one of the “big 5” CYPs (*CYP1A2*, *CYP2C9*, *CYP2C19*, *CYP2D6*, and *CYP3A*), which collectively are responsible for $\approx 90\%$ of the oxidative metabolism of drugs.¹ Caffeine is a widely used and sensitive *in vivo* *CYP1A2* index substrate because of rapid and nearly complete absorption into the systemic circulation upon oral administration (oral bioavailability, $\sim 99\%$) and extensive hepatic metabolic clearance.^{2–4} *CYP1A2*–mediated

demethylation of caffeine to the primary metabolite, paraxanthine, represents $>80\%$ of total caffeine clearance; other primary clearance pathways include demethylation to theobromine and theophylline (Figure 1).^{5,6} The caffeine metabolic ratio, based on plasma concentration at a single timepoint or area under the concentration–time curve (AUC) and of the mass excreted into urine over a certain time interval, is a commonly used marker of *CYP1A2* activity.^{7–10}

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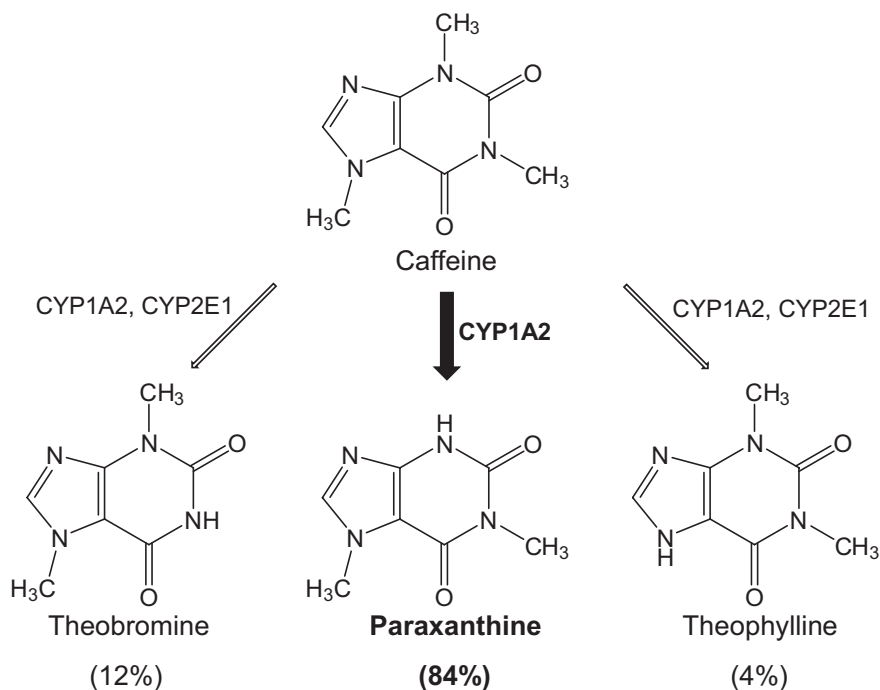


Figure 1 Primary metabolic pathways of caffeine.

As with all CYP index substrates, robust estimates of both interindividual and intraindividual variation in CYP1A2 activity are needed to determine optimal sample size for clinical pharmacokinetic xenobiotic–caffeine interaction studies.^{4,11} Multiple factors contribute to the large interindividual variation in CYP1A2 activity (5- to 15-fold) among various populations¹² and include sex (activity generally higher in men compared with women), smoking (CYP1A2 inducer), concomitant medications (e.g., oral contraceptives, which are CYP1A2 inhibitors), and genetic polymorphisms. Common CYP1A2 haplotypes include CYP1A2*1A, CYP1A2*1C, CYP1A2*1F, and CYP1A2*1K.^{12,13} CYP1A2*1A is considered the reference haplotype. CYP1A2*1C is defined by the single-nucleotide polymorphism (SNP) –3860G>A (*rs2069514*); CYP1A2*1F is defined by the SNP –163C>A (*rs762551*); and CYP1A2*1K is defined by the SNPs –739T>G (*rs2069526*), –729C>T (*rs12720461*), and –163C>A (*rs762551*).^{13,14} Haplotype frequencies vary widely with ethnicity. For example, the frequency of CYP1A2*1C ranges from 0.4% to 1% in whites to 7% in blacks or African Americans to 6–25% in Asians,¹⁴ whereas that for CYP1A2*1F ranges from ≥0.5% in whites and African Americans to <8% in Asians.^{15,16}

Compared with CYP1A2*1A, CYP1A2*1C and CYP1A2*1K are associated with decreased CYP1A2 activity *in vivo*. CYP1A2*1F is generally associated with increased activity because of increased inducibility or “hyperinducibility.”¹³ For example, both smoking^{17–20} and heavy caffeine consumption (≥3 cups/day)²¹ were associated with higher CYP1A2 activity (by 30–70%) in CYP1A2*1F*1F carriers vs. CYP1A2*1A*1F and CYP1A2*1A*1A carriers. Compared with the interindividual variation, published studies with respect to the intraindividual variation in CYP1A2 activity are few (Table 1). These studies collectively addressed the effects of

smoking status, age, and sex using either urinary or plasma ratios at a single timepoint. The effects of common genetic CYP1A2 polymorphisms and use of oral contraceptives by women were not considered. In addition, caffeine consumption habit was not reported.

Caffeine, as brewed coffee, carbonated beverage, and energy drink, is heavily consumed worldwide. Approximately 50% of adults in the United States alone consume 1–3 cups of coffee/day.^{22,23} Based on safety concerns with energy drinks and other nonconventional forms of caffeine, a previous study compared the pharmacokinetics of caffeine under different drink temperature (hot vs. cold), rate of consumption (2 vs. 20 minutes), and vehicle (coffee vs. energy drink) conditions.²⁴ Each of 24 nonsmoking, non-naïve, caffeine-drinking (1–3 cups/day) volunteers was administered 160 mg caffeine, as coffee or energy drink, under the various conditions. The pharmacokinetics of caffeine did not differ among conditions, indicating no significant impact of temperature, consumption rate, and vehicle.²⁴ Based on the aforementioned gaps in the literature, the objective of the present study was to assess the effects of CYP1A2 induction status (based on genotype), sex, and oral contraceptive use by women on both the interindividual and intraindividual variation in the caffeine metabolic ratio. Plasma (0–8 hours) and DNA were obtained from this well-characterized cohort of participants. Results from this exploratory analysis provide a robust estimate of intraindividual variation in CYP1A2 activity for optimal design of clinical xenobiotic–caffeine interaction studies.

METHODS

Pharmacokinetic study

Plasma and whole blood were obtained from a previous study involving 24 healthy volunteers (12 men) who were

Table 1 Intraindividual variability in the caffeine metabolic ratio reported in the literature

Race/ ethnicity ^a	Sex	Smoker?	Age (y)		Caffeine formulation (caffeine content)	Metabolic ratio		No. of replicates within a subject	Interval of replicate measurement	CV (%)	
			Mean ± SD	No. of subjects		Description	Mean ± SD			Mean (range)	Reference
Japanese	M	No	30.4 (17–46) ^b	5	Instant coffee (caffeine content NS)	Urinary (17U, PX)/ CAF, 4–5 h collection interval	8.7	2	11 mo	27.5 (0.7–66.7)	31
	F	No	31.7 (23–36) ^b	3			2.8			59.5 (41.6–84.5)	
White	M	Yes	35.3 (25–50) ^b	4			11.6			17.6 (6.9–37.6)	
	M	No	(22–61) ^b	10	Brewed coffee (~200 mg)	Urinary PX/CAF, 4–5 h collection interval	4.5 ± 1.7	3	1 wk	24.3 (3.0–71.5)	28
	F	No		9			5.5 ± 1.5			26.2 (4.2–60.4)	
NS	M	No	34.8 ± 7.9	10	NoDoze (2 mg/ kg)	Urinary (1U+1X+AFMU)/17U, 14–15 h collection interval	9.4 ± 10.2	6		18.6 (4.5–45.3)	29
	F	No	38.2 ± 9.3	10			5.7 ± 2.3			20.7 (8.7–49.3)	
NS	M	No	Young subjects	34	Koffein "Dak" (200 mg)	Plasma PX/CAF at 6 h	0.75 ± 0.22	2		18 (1–45)	32
	M	Yes		8			1.6 ± 0.7		12–14 wk	16 (2–46)	
NS	NS	No	25 ± 0.3	12	Instant coffee (150 mg)	Plasma PX/CAF at 5 h	0.45 ± 0.19	5	3 wk	16.2 (6.9–25.7)	33
		Yes		4			0.76 ± 0.21			21.8 (18.9–26.5)	
		No	70 ± 1.7	13			0.32 ± 0.14			16.4 (8.6–32.9)	
		Yes		3			0.45 ± 0.07			15.3 (10.4–18.5)	

AFMU, 5-acetylamino-6-formylamino-3-methyluracil; CAF, caffeine; CV, coefficient of variation; F, female; M, male; NS, not specified; PX, paraxanthine; 1U, 1-methyl uric acid; 17U, 1,7-dimethyluric acid; 1X, 1-methylxanthine. ^aThe studies from references 29, 32, and 33 were conducted in the United States, Denmark, and France, respectively. ^bMean (range) or (range).

Table 2 CYP1A2 induction status and genotype for 24 healthy volunteers

Induction Status	Genotype	SNP ^a		Sex (n)	Race (n)
		-3860G>A	-163C>A		
N	*1A/*1A	GG	CC	M (1), F (3)	White (3), Asian (1)
N	*1C/*1F	GA	CA	M (1), F (1)	Asian (1), PI (1)
N	*1C*1F/*1C*1F	AA	AA	M (2), F (1)	Asian (2), PI (1)
H	*1A/*1F	GG	CA	M (6), F (5)	White (9), Asian (2)
H	*1F/*1F	GG	AA	M (2), F (2)	White (4)

F, female; H, hyperinducer; M, male; N, normal metabolizer; SNP, single-nucleotide polymorphism; PI, Pacific Islander.

^aAll subjects harbored the T allele for -739T>G (*rs2069526*) and the C allele for -729C>T (*rs12720461*).

nonsmoking, non-naïve caffeine drinkers (1–3 caffeine-containing beverages/day on average), aged 18 to 30 years; 16 subjects were self-identified as white, six as Asians, and two as Pacific Islanders.²⁴ In brief, the pharmacokinetics of a single oral dose of caffeine (160 mg), administered in the form of instant coffee or a sugar-free energy drink, were compared with respect to temperature and rate of consumption: arm A, hot coffee consumed over 20 minutes; arm B, cold coffee consumed over 2 minutes; arm C, cold coffee consumed over 20 minutes; arm D, energy drink consumed over 2 minutes; and arm E, energy drink consumed over 20 minutes. Study arms were separated by at least 3 days. Before the first treatment, subjects were asked to abstain from caffeine ~24 hours before caffeine administration. DNA was extracted from whole blood using the QIAamp DNA blood mini kit (Qiagen, Germantown, MD) and stored at -80°C pending analysis for the CYP1A2 SNPs *rs2069514*, *rs2069526*, *rs762551*, and *rs12720461* by Genelex Labs (Seattle, WA).

Analysis of plasma for caffeine and paraxanthine

Plasma was analyzed for caffeine as described using a Xevo G2S QToF mass spectrometer (Waters, Millford, MA) coupled to an ultra-high-pressure liquid chromatographic system (Waters).²⁴ Plasma was analyzed for paraxanthine in a similar manner using d₃-paraxanthine (Toronto Research Chemicals, Toronto, ON, Canada) as the internal standard. Analytes were separated via an Acquity HSS T3 column (2.1 × 100 mm, 1.8 μm) (Waters). The mass spectrometer was operated in positive ion mode using *m/z* values of 181.070 and 184.091 for paraxanthine and d₃-paraxanthine, respectively. The limit of quantification was 39 ng/mL.

Pharmacokinetic and statistical analysis

The pharmacokinetics of caffeine were determined via a noncompartmental methods as described²⁴ using Phoenix WinNonlin, version 6.4 (Certara, St. Louis, MO). The concentrations of caffeine and paraxanthine at 4 hours (C_{4h,CAF} and C_{4h,PX}, respectively) were obtained directly from the plasma concentration–time profiles. Areas under the curves from time 0 to 8 hours for caffeine and paraxanthine (AUC_{0–8h,CAF} and AUC_{0–8h,PX}, respectively) were calculated using the trapezoidal method with linear interpolation. Caffeine metabolic ratios based on C_{4h} and AUC_{0–8h} were calculated using molar concentrations.

Caffeine metabolic ratios were log transformed before statistical analysis. Repeated-measures analysis of variance was used to test for differences in the mean C_{4h,PX}/C_{4h,CAF} and AUC_{0–8h,PX}/AUC_{0–8h,CAF} ratios among the five treatment arms; *P* < 0.05 was considered significant. The 24 subjects were grouped with respect to sex, CYP1A2 induction status, and oral contraceptive use by six of the women to make comparisons. Within each study arm, the Wilcoxon-Mann-Whitney *U*-test was used to test for differences in mean C_{4h,PX}/C_{4h,CAF} and AUC_{0–8h,PX}/AUC_{0–8h,CAF} ratios; *P* < 0.05 was considered significant. Interindividual variability in CYP1A2 activity was calculated as the coefficient of variation (CV), the ratio of SD/mean, for each metabolic ratio within a treatment arm. Intraindividual variability in CYP1A2 activity was calculated as the CV for each ratio among the five treatment arms within each subject. Differences in interindividual and intraindividual variability between two groups were compared using the Wilcoxon-Mann-Whitney *U*-test; *P* < 0.05 was considered significant.

RESULTS

CYP1A2 genotype

Using the PharmGKB summary for CYP1A2 as a guide,¹³ subjects with genotypes CYP1A2*1F/*1F and CYP1A2*1A/*1F were designated hyperinducers (*n* = 15), and subjects with genotypes CYP1A2*1A/*1A, CYP1A2*1C/*1F, or CYP1A2*1C*1F/*1C*1F were designated normal metabolizers (*n* = 9) (Table 2).

Caffeine metabolic ratios

The pharmacokinetics of caffeine were consistent with those determined in the previous study in which a different pharmacokinetic analysis program was used.²⁴ Paraxanthine concentrations increased, reaching a plateau at ~4 hours that remained until the last plasma collection at 8 hours (data not shown). Plasma caffeine metabolic ratios based on C_{4h} and AUC_{0–8h} ranged from 0.12–0.96 and 0.12–0.79, respectively, with all treatment arms and subjects combined (*n* = 120 total measurements for each ratio); there was a strong correlation between the two ratios (*r* = 0.97). The mean C_{4h} ratios did not differ among treatment arms with all subjects combined (0.37–0.41). When stratified by sex, the C_{4h} ratio was significantly higher in men, by ~40%, compared with women (*P* < 0.01) with all subjects and treatment arms combined; if the six women taking oral contraceptives were excluded, the difference was 24% (*P* < 0.03) (Table 3). Within each treatment arm, a

Table 3 Caffeine metabolic ratios and interindividual variability within each treatment arm

Treatment arm	Metabolic ratio (mean ± SD)		CV (%)	
	C _{4 h,PX} /C _{4 h,CAF}	AUC _{0–8 h,PX} /AUC _{0–8 h,CAF}	C _{4 h,PX} /C _{4 h,CAF}	AUC _{0–8 h,PX} /AUC _{0–8 h,CAF}
Men (n = 12)				
A	0.44 ± 0.14	0.40 ± 0.13	31.3	31.5
B	0.44 ± 0.12	0.39 ± 0.10	26.5	24.8
C	0.48 ± 0.13	0.45 ± 0.14	27.6	31.2
D	0.46 ± 0.16	0.42 ± 0.14	34.7	33.1
E	0.50 ± 0.17	0.45 ± 0.11	32.9	25.4
All arms ^a	0.46 ± 0.14	0.42 ± 0.12	30.6 ± 3.50	29.2 ± 3.82
Women (n = 12)				
A	0.32 ± 0.12	0.30 ± 0.10	37.5	32.7
B	0.30 ± 0.13	0.28 ± 0.10	44.5	37.3
C	0.31 ± 0.15	0.30 ± 0.13	47.3	44.3
D	0.32 ± 0.16	0.29 ± 0.11	51.2	38.8
E	0.33 ± 0.13	0.32 ± 0.11	40.4	35.9
All arms ^a	0.32 ± 0.14 ^b	0.30 ± 0.11 ^b	44.2 ± 5.44 ^c	37.8 ± 4.28 ^c
OC users (all arms) ^a	0.26 ± 0.11 ^d	0.25 ± 0.09 ^d	44.8 ± 22.8	41.5 ± 14.6
Non-OC users (all arms) ^a	0.37 ± 0.14 ^e	0.34 ± 0.11 ^e	39.1 ± 5.44	33.0 ± 3.45

AUC, area under the concentration–time curve; CAF, caffeine; CV, coefficient of variation; OC, oral contraceptive; PX, paraxanthine.

^aData represent mean ± SD of five arms. ^b*P* < 0.01 compared with men (Wilcoxon-Mann-Whitney *U*-test). ^c*P* < 0.02 compared with men (Wilcoxon-Mann-Whitney *U*-test). ^d*P* < 0.0001 compared with men (Wilcoxon-Mann-Whitney *U*-test). ^e*P* < 0.03 compared with men (Wilcoxon-Mann-Whitney *U*-test).

sex difference was detected (*P* < 0.05) (Table 3, Figure 2) but was not detected if the oral contraceptive users were excluded. Within women in each treatment arm, the C_{4 h} ratio tended to be lower in oral contraceptive users compared with nonusers (*P* > 0.07). The mean interindividual CV was significantly lower in men, by 44%, compared with women (*P* < 0.02); a sex difference was not detected if the oral contraceptive users were excluded (Table 3). Comparisons using the AUC_{0–8 h} ratio were similar to those using the C_{4 h} ratio.

Because the metabolic ratio did not differ between the various conditions, intraindividual variation in CYP1A2 activity was calculated using the metabolic ratio obtained from the five arms for each subject. A sex difference was not detected in intraindividual CV for the C_{4 h} ratio (Table 4). Within the 16 white subjects, the intraindividual CV for the C_{4 h} ratio was comparable between men and women (data not shown). When stratified by induction status based on CYP1A2 genotype (hyperinducer or normal metabolizer), the C_{4 h} ratio did not differ with all subjects and treatment arms combined (data not shown) and within each treatment arm (Figure 3). The intraindividual CV for the C_{4 h} ratio did not differ between hyperinducers and normal metabolizers for all subjects (Table 4) and for white subjects (data not shown). Comparisons using the AUC_{0–8 h} ratio were similar to those using the C_{4 h} ratio except the AUC-based intraindividual CV within the 16 white subjects was significantly lower in men, by 44%, compared with women (*P* = 0.03; data not shown).

DISCUSSION

Caffeine, consumed worldwide as a beverage, is a sensitive index CYP1A2 substrate⁴ commonly used to evaluate the CYP1A2 inhibitory or inductive effects of new molecular

entities and other xenobiotics, including herbal and other natural products.^{25,26} The optimal sample size calculation for clinical pharmacokinetic xenobiotic–drug interaction studies requires robust estimates of both interindividual and intraindividual variation in the primary end point, which for CYP-mediated interactions typically involves a validated urinary or plasma metabolic ratio of the index substrate. Sex, smoking status, concomitant oral contraceptive use by women, and genetic polymorphisms are established factors that influence variation in the caffeine metabolic ratio.

Relative to estimates of interindividual variation, estimates of intraindividual variation in the caffeine metabolic ratio are few (Table 1). In addition, none of those studies assessed the effects of CYP1A2 induction status and oral contraceptive use by women and did not report whether the subjects were habitual caffeine consumers, the latter of which reflect at least half of the US and other populations.^{22,23,27} Based on these knowledge gaps, the objective of this study was to revisit the intraindividual variation in the caffeine metabolic ratio by assessing the effects of CYP1A2 induction status (based on genotype) and other key factors. The analysis involved plasma and DNA specimens obtained from a previous study involving 24 non-smoking, non-naïve, caffeine-drinking healthy volunteers whose caffeine pharmacokinetics were determined on five separate occasions.²⁴ Because the pharmacokinetics did not differ under the various conditions (drink temperature, consumption time, and caffeine vehicle), this cohort of subjects was representative of a major segment of the population.

Among the 24 subjects, the caffeine metabolic ratio, based on either C_{4 h} or AUC_{0–8 h}, varied from four- to six fold within each treatment arm. This interindividual variability was near the low end of the 5- to 15-fold reported

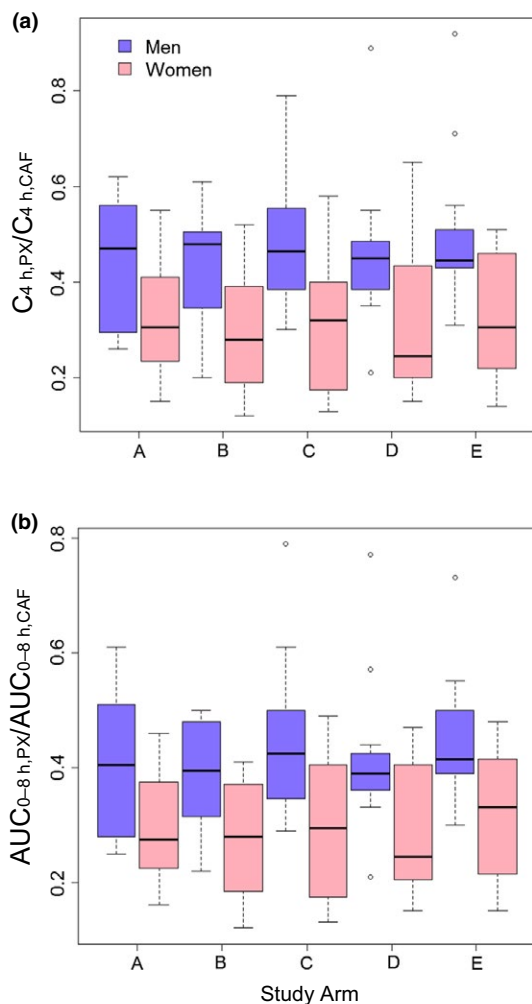


Figure 2 Box-and-whisker plots of $C_{4h,PX}/C_{4h,CAF}$ (a) and $AUC_{0-8h,PX}/AUC_{0-8h,CAF}$ (b) ratios for each study arm among men and women. Arm A, hot coffee consumed over 20 minutes; arm B, cold coffee consumed over 2 minutes; arm C, cold coffee consumed over 20 minutes; arm D, energy drink consumed over 2 minutes; and arm E, energy drink consumed over 20 minutes. Lines inside the boxes denote medians, ends of boxes denote 25th and 75th percentiles, whiskers denote 1.5 times the interquartile distance, and circles denote outliers. A sex difference was detected ($P < 0.05$; Wilcoxon-Mann-Whitney *U*-test) within each treatment arm when oral contraceptive-using women were included.

range,¹² which likely reflected the relatively homogeneous nature of the study subjects. When stratified by sex, the mean metabolic ratios were higher in men compared with women, by ~40% (Table 3). Oral contraceptive use by women, which has been reported to inhibit CYP1A2 activity,^{15,17} may contribute to this sex difference. In the current study, oral contraceptive users tended to have lower metabolic ratios compared with nonusers, which further explains the observation in the previous study²⁴ that caffeine apparent oral clearance was lower in oral contraceptive users compared with nonusers (0.8 vs. 1.3 mL/min per kg). If the nonusers were excluded from the analysis, a sex difference was not detected within each treatment arm, consistent with a previous report.¹⁷ Interindividual

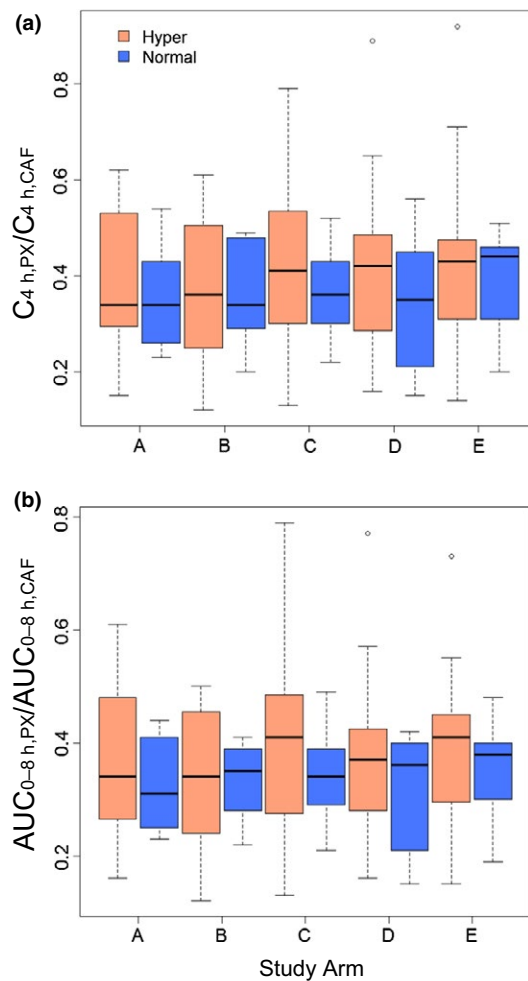


Figure 3 Box-and-whisker plots of $C_{4h,PX}/C_{4h,CAF}$ (a) and $AUC_{0-8h,PX}/AUC_{0-8h,CAF}$ (b) ratios for each study arm among CYP1A2 hyperinducers (Hyper) and normal metabolizers (Normal). Arm A, hot coffee consumed over 20 minutes; arm B, cold coffee consumed over 2 minutes; arm C, cold coffee consumed over 20 minutes; arm D, energy drink consumed over 2 minutes; and arm E, energy drink consumed over 20 minutes. Lines inside the boxes denote medians, ends of boxes denote 25th and 75th percentiles, whiskers denote 1.5 times the interquartile distance, and circles denote outliers. No differences were detected within each treatment arm ($P > 0.05$; Wilcoxon-Mann-Whitney *U*-test).

variability was greater in women compared with men (~40% vs. 30%) (Table 3); again, if the oral contraceptive users were excluded, a difference was not detected. Although in agreement with the previous study that involved a much larger sample size (>200 subjects),¹⁷ these observations contradict those from two other equally large studies showing that oral contraceptive use did not contribute to the sex difference in CYP1A2 activity. Reasons for these discrepancies may include a relatively understudied ethnic group with respect to variation in CYP1A2 activity (Turkish)¹⁸ and enrollment of smokers only,¹⁹ the latter of which suggests that smoking may mask the CYP1A2 inhibitory effects of oral contraceptives.

The intraindividual variability in the caffeine metabolic ratio was assessed by the CV, which was calculated from

Table 4 Intraindividual variability in caffeine metabolic ratio stratified by sex, oral contraceptive use by women, induction status, and race

Subjects ^a	No. of subjects	CV (%) (mean ± SD)	
		$C_{4\text{ h,PX}}/C_{4\text{ h,CAF}}$	$AUC_{0-8\text{ h,PX}}/AUC_{0-8\text{ h,CAF}}$
Men	12	19.1 ± 5.7	17.1 ± 6.9
Women	12	26.4 ± 16.6	23.1 ± 12.4
Women using OCs	6	34.6 ± 19.0	30.1 ± 13.2
Women not using OCs	6	18.3 ± 9.1	16.2 ± 7.1
Hyperinducers	15	25.8 ± 14.7	23.2 ± 11.2
Normal metabolizers	9	17.8 ± 6.2	15.1 ± 6.2
White subjects	16	24.3 ± 15.0	20.8 ± 12.0
Nonwhite subjects	8	19.8 ± 5.7	18.9 ± 6.2
All subjects	24	22.8 ± 12.7	20.1 ± 10.3

AUC, area under the concentration–time curve; CAF, caffeine; CV, coefficient of variation; OC, oral contraceptive; PX, paraxanthine.

^aA significant difference was not detected between subjects when stratified by sex, OC use, induction status, and race.

the five replicates obtained from each subject. Among all subjects, the CV was ≈20% (**Table 4**). When stratified by sex, induction status, oral contraceptive use by women, or race, the CV was generally no more than 30%; the only exception was the $C_{4\text{ h}}$ -based CV of 35% in women taking oral contraceptives. Although the value was higher in women compared with men (~25% vs. 18%), a sex difference was not detected, even if extreme values for each sex were excluded. Similar trends were observed for the white subjects (data not shown). These data are consistent with previous reports involving nonsmoking subjects, most presumed to be white^{28,29} (**Table 1**). Within women, use of oral contraceptives did not affect intraindividual CV significantly. With respect to CYP1A2 induction status, the mean metabolic ratios did not differ between hyperinducers and normal metabolizers (~0.39 vs. ~0.35; **Figure 3**), yet the intraindividual CV was higher in hyperinducers compared with normal metabolizers (25% vs. 16%; **Table 4**). Similar trends were observed comparing only the homozygous CYP1A2*1F carriers with noncarriers (data not shown). These data indicate that common CYP1A2 genotypes are not robust predictors of CYP1A2 phenotype (**Table 2**), consistent with previous reports.^{10,21}

Heavy coffee consumption (≥3 cups/day) was shown to be associated with higher CYP1A2 activity in nonsmoking white subjects.²¹ The previous studies (**Table 1**) did not report caffeine consumption habit; thus, the effects of caffeine consumption on the intraindividual CV could not be assessed. All subjects in the current work were known to be non-naïve caffeine drinkers (1–3 cups/day). Because the CV was similar to that for the previous studies, moderate caffeine consumption is an unlikely contributor to intraindividual variation in CYP1A2 activity.

In addition to the aforementioned factors that could contribute to the intraindividual variation in the caffeine metabolic ratio, other factors, such as the time interval

between replicate measurements, plasma vs. urinary ratios, age, and smoking status, likely contribute. However, with the exception of the one study involving a few Japanese subjects, the intraindividual CV reported in the previous studies was always <30% (**Table 1**). This observation, combined with those in the current study (**Table 4**), suggest 30% intraindividual variation may be the most appropriate for optimal sample size calculation for CYP1A2-mediated xenobiotic–caffeine interaction studies.

There are limitations to the current work. First, because this study was exploratory, firm conclusions cannot be made about the effects of CYP1A2 induction status, sex, and oral contraceptive use by women on the intraindividual variation in the caffeine metabolic ratio. Based on the current data (**Table 4**) and assuming a type I error of 0.05 and a power of 0.80, 33 and 21 subjects per group would be needed to detect a difference in intraindividual variation based on $C_{4\text{ h,PX}}/C_{4\text{ h,CAF}}$ and $AUC_{0-8\text{ h,PX}}/AUC_{0-8\text{ h,CAF}}$, respectively, between hyperinducers and normal metabolizers. Second, a limited number of CYP1A2 genotypes were determined. Forty-one CYP1A2 haplotypes have been reported to date;¹³ thus, investigation of other haplotypes is warranted to improve the understanding of the CYP1A2 genotype–phenotype relationship. Third, most study subjects were self-identified as white. CYP1A2 activity has been reported to be higher in white individuals compared with Asians and African Americans.^{17,30} Whether different frequencies of multiple genotypes across race/ethnicity influence intraindividual variation remains to be determined. Fourth, although CYP1A2 activity did not differ among the different treatment conditions, drink temperature, consumption rate, and vehicle may have confounded the assessment of intraindividual variability.

In summary, this exploratory study demonstrated that the intraindividual variation in CYP1A2 activity in a well-characterized group of young nonsmoking, non-naïve, caffeine-drinking healthy volunteers was generally no more than 30%, regardless of CYP1A2 induction status, sex, and oral contraceptive use by women. These results add to information from previous studies (**Table 1**) in which intraindividual variability in CYP1A2 activity, stratified by smoking status, sex, and age, was generally <30%. Collectively, a value of 30% is proposed for optimal design of clinical pharmacokinetic xenobiotic–caffeine interaction studies. Prospective studies are needed to confirm these observations.

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