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A Pharmacometric Approach to Investigate the Impact of Methylxanthine Abstinence and Caffeine Consumption on CYP1A2 Activity

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ABSTRACT

This study aimed to investigate the impact of methylxanthine abstinence (MA) periods on CYP1A2 activity in individuals with varying levels of caffeine consumption through development of a population pharmacokinetic model of caffeine and its major metabolite paraxanthine. This study developed and evaluated a mixed-effects pharmacokinetic model for caffeine and paraxanthine concentration-time data derived from a sequential single-dose cross-over study in healthy male volunteers (*n* = 30) who received oral 100 mg caffeine doses. Participants received caffeine with and without a MA period. Participants were classified as low (0–100 mg/d), medium (100–200 mg/d), or high (>200 mg/d) caffeine consumers (LCCs, MCCs, or HCCs, respectively). All caffeine and paraxanthine concentration-time data were simultaneously modeled. Caffeine pharmacokinetics was described by a two-compartment model with first-order absorption and two first-order elimination pathways. Paraxanthine was described by a one-compartment model with first-order absorption and elimination. Among LCCs (*n* = 16) and MCCs (*n* = 9), there was no difference in the mean (95% confidence interval) total apparent caffeine clearance (CL) between the MA period [LCCs: 6.88 (5.61–8.16 l/h); MCCs: 10.09 (7.57–12.60 l/h)] versus the no MA period [LCCs: 6.22 (4.97–7.46 l/h); MCCs: 9.68 (7.12–12.24 l/h)]. The mean CL among HCCs (*n* = 5) was considerably higher in the MA period [10.48 (5.62–15.33 l/h)] compared with the no MA period [6.30 (3.40–9.20 l/h)] (*P* < 0.05). The decrease in CL in the no MA period among HCC appears to be due to alternative caffeine elimination pathways, rather than CYP1A2.

Introduction

The CYP1A2 enzyme contributes to the metabolism of approximately 11% of all therapeutically used medicines, including olanzapine, clozapine, and theophylline (Faber et al., 2005; Perera et al., 2013b). CYP1A2 activity demonstrates a large degree of variability in human populations, with a 10- to 15-fold variation reported in vivo (Aklillu et al., 2003; Djordjevic et al., 2008; Perera et al., 2012a). Studies have attempted to uncover factors that contribute to CYP1A2 variability, including genetic variants, in humans through the use of in vivo phenotyping utilizing caffeine as a probe drug (Aklillu et al., 2003; Ghotbi et al., 2007; Djordjevic et al., 2008; Perera et al., 2012a, 2013a). There is also increasing interest in the use of the CYP1A2 phenotype to individualize the dose of drugs metabolized by this enzyme pathway; therefore, it is essential to ensure that an accurate estimate of CYP1A2 activity is obtained (Perera et al., 2013b).

Caffeine (1,3,7-trimethylxanthine) is currently the most widely accepted probe used in vivo to investigate the activity of CYP1A2 in humans (Perera et al., 2012b). In vitro studies, which do not account for renal elimination, indicate that caffeine is biotransformed to paraxanthine (1,7-dimethylxanthine), theobromine (3,7-dimethylxanthine), and theophylline (1,3-dimethylxanthine), which account for 83.4, 11.1, and 5.5% of total caffeine metabolism, respectively (Fig. 1) (Gu et al., 1992; Kalow and Tang, 1993). The pathway forming paraxanthine is exclusively mediated by CYP1A2, whereas CYP2E1 and CYP3A4 play a role in the formation of theophylline and theobromine (Butler et al., 1992; Gu et al., 1992). In vivo studies report similar findings based on changes in caffeine exposure resulting from coadministration of CYP1A2 inhibitors (e.g., furafylline) (Tarrus et al., 1987; Sesardic et al., 1990).

Apparent caffeine clearance is considered to be a gold standard measurement of CYP1A2 activity (Kalow and Tang, 1993). However, on the basis of the exclusive metabolism of caffeine to paraxanthine by CYP1A2, the ideal measurement would be the apparent clearance of

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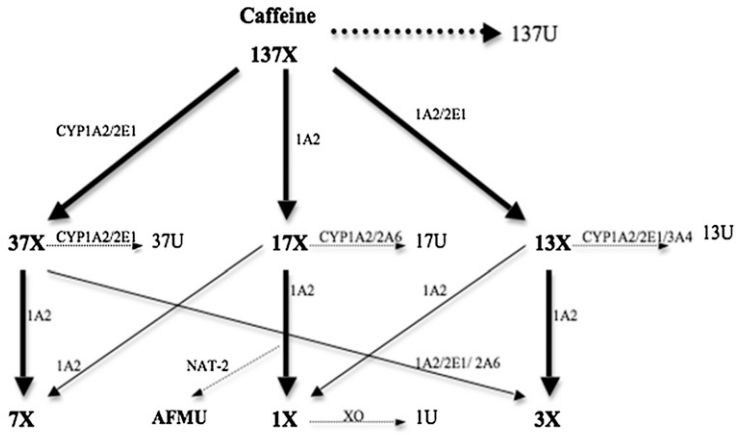
ABBREVIATIONS: AIC, Akaike's information criterion; AUC, area under the curve; CL, clearance; HCC, high caffeine consumer; LCC, low caffeine consumer; LOQ, limit of quantification; MA, methylxanthine abstinence; MCC, medium caffeine consumer; MM, Michaelis-Menten; NCA, noncompartmental analysis; RSE%, relative standard error as a percentage.



137X (Caffeine): 1,3,7 –trimethylxanthine: 137U: 1,3,7-trimethyluric acid: 17X: paraxanthine: 17U: 1,7-methyluric acid: 37X: theobromine: 37U: 3,7-methyluric acid: 13X: theophylline: 13U: 1,3-methyluric acid: 7X: 7-methylxanthine: 1X: 1-methylxanthine: 3X: 3-methylxanthine: XO: xanthine oxidase: AFMU: 5-acetylamino-formylamino-3-methyluracil: CYP1A2 (1A2): cytochrome P-4501A2: CYP2E1 (2E1): cytochrome P-4502E1: CYP2A6 (2A6): cytochrome P-4502A6: CYP3A4 (cytochrome P-4503A4.

Fig. 1. Involvement of human cytochrome P450 enzymes in the biotransformation of caffeine (137X) and its metabolites, as indicated by experiments using HepG2 cells and expressed cDNA (adapted from Gu et al., 1992). Dashed lines indicate hydroxylation pathways, whereas solid lines represent demethylation pathways. 1X, 1-methylxanthine; 3X, 3-methylxanthine; 7X, 7-methylxanthine; 13U, 1,3-methyluric acid; 13X, theophylline; 17U, 1,7-methyluric acid; 17X, paraxanthine; 37U, 3,7-methyluric acid; 37X, theobromine; 137U, 1,3,7-trimethyluric acid; AFMU, 5-acetylamino-formylamino-3-methyluracil; XO, xanthine oxidase.

caffeine to paraxanthine (Tucker et al., 1998). Typically, this is not a feasible measurement when noncompartmental pharmacokinetic methods are used unless paraxanthine is directly administered. Pharmacometric tools allow modeling of concentration-time data using prior knowledge of the pathways associated with metabolism and thus can be used to generate estimates of clearance not usually obtained experimentally (Rostami-Hodjegan et al., 1996; Bulitta et al., 2010).

Previous studies have indicated that at high or repeated doses, caffeine displays nonlinear (dose-dependent) pharmacokinetics (Bonati et al., 1982; Blanchard and Sawers, 1983; Renner et al., 1984; Cheng et al., 1990; Denaro et al., 1990, 1991). Because CYP1A2 is considered to be the primary enzymatic pathway for caffeine elimination, it is assumed that saturation of this metabolic pathway is the cause of the observed dose dependency; however, the mechanism for the change in apparent caffeine clearance has not been established (Arnaud, 2011). In vivo and in vitro studies have indicated that regular caffeine consumption can induce CYP1A2 activity (Berthou et al., 1995a; Perera et al., 2012a). Many in vivo studies have applied a methylxanthine abstinence (MA) period prior to CYP1A2 phenotyping to ensure complete washout of caffeine to conduct noncompartmental analysis (NCA) (Tantcheva-Poór et al., 1999; Aklillu et al., 2003; Ghotbi et al., 2007; Djordjevic et al., 2008). However, these studies have not considered the potential impact of MA on the pharmacokinetics or pharmacodynamics of caffeine and its metabolites, thereby altering the obtained caffeine metric.

Investigating the underlying mechanism responsible for the observed changes in the pharmacokinetics of caffeine and the impact of a MA period is an important consideration to ensure that the accurate CYP1A2 phenotype is obtained. This has implications for CYP1A2 phenotype-genotype associations and for dose individualization of CYP1A2 metabolized medicines. The aim of this study was to investigate the impact of MA periods in individuals with varying levels of caffeine consumption on CYP1A2 activity by developing a population pharmacokinetic model of caffeine and its major metabolite paraxanthine.

Materials and Methods

Patient Population and Study Design. Caffeine and paraxanthine concentration-time data were obtained from a previous study that investigated CYP1A2 metrics in plasma and saliva in 30 subjects (Perera et al., 2011). However, the previous study used NCA to calculate pharmacokinetic parameters, namely apparent caffeine clearance, in the MA period was unable to obtain pharmacokinetic parameters in the no MA period due to the assumptions associated with NCA. Pharmacometric modeling allows the calculation of these pharmacokinetic parameters without the assumptions of NCA.

Study participants were healthy nonsmoking male volunteers of South Asian or European ancestry who had a mean (± S.D.) age of 24.0 ± 4.7 years, weight of 78.5 ± 11.4 kg, and body mass index of 24.5 ± 2.8 kg/m^2. Each participant received a 100 mg caffeine tablet (NoDoz tablets; Key Pharmaceutical, North Ryde, NSW, Australia) on two occasions. In the first phase of the study, participants were asked to abstain from any methylxanthine-containing foods and beverages for 24 hours prior to ingestion of the caffeine study dose, whereas in the second phase of the study, participants were asked to maintain their usual methylxanthine intake prior to administration of the study dose (Perera et al., 2011). The participants were observed between 0 and 10 hours of the study. They were not observed during 10–24 hours; therefore, it is possible that the participants would have consumed caffeine in this period. However, we found no deviations in the 24-hour concentration range that were outside the range of the typical values (based on more than 3 S.D. from the mean) and none were considered outliers from visual inspection of the pharmacokinetic profiles.

Sampling Times and Sample Analysis. In both study phases, blood samples (approximately 10 ml) were collected via an indwelling cannula at 5 minutes prior to administration of the study dose and at 0.5, 1, 1.5, 2, 4, 6, 8, 10, and 24 hours after the study dose. After centrifugation, plasma was harvested and stored at −20°C. Caffeine and paraxanthine concentrations were quantified after liquid-liquid extraction and a selective validated high-performance liquid chromatography assay (Perera et al., 2010). The intraday and interday coefficients of variation of the assay were less than 15%, whereas the intraday and interday accuracy of the assay was greater than 86% for both caffeine and paraxanthine. The limit of quantification (LOQ) in plasma was 0.025 μg/ml and the limit of detection was 0.01 μg/ml for both analytes (Perera et al., 2011).

Data Collection and Caffeine Intake. Information was collected regarding each participant's diet, specifically relating to methylxanthine, including caffeine intake. The type of caffeine intake and frequency per day were recorded for each participant. Daily caffeine intake for each participant was calculated according to Food Standards Australia New Zealand guidelines (Smith et al., 2000). The time of last caffeine ingestion before the caffeine dose was recorded. Participants were classified as low (caffeine intake 0–100 mg/d), medium (100–200 mg/d), and high (>200 mg/d) caffeine consumers (LCCs, MCCs, and HCCs, respectively) (Perera et al., 2012a). There were 16 LCCs with a caffeine intake less than 100 mg/d (range, 0–75 mg/d), 9 MCCs with caffeine intake between 100 and 200 mg/d (range, 110–155 mg/d), and 5 HCCs with caffeine intake above 200 mg/d (range, 205–340 mg/d) (Table 1).

Noncompartmental Pharmacokinetic Methods. The apparent caffeine clearance in the 24-hour MA phase for each of the study participants was

TABLE 1

Demographics of participants stratified by caffeine consumption

Data are presented as the median (range from minimum to maximum).

Demographic	HCCs	MCCs	LCCs
Participants (*n*)	5	9	16
Age (yr)	23.0 (21.0–33.0)	23.0 (19.0–32.0)	24.0 (19.0–42.0)
Weight (kg)	82.9 (65.0–108.0)	79.5 (64.9–108.0)	75.0 (60.0–89.0)
Height (m)	1.86 (1.67–1.92)	1.79 (1.65–1.99)	1.76 (1.67–1.90)
Daily caffeine intake (mg/d)	240.0 (205.0–340.0)	120.0 (110–155.0)	60.0 (0.0–75.0)

analyzed by NCA using Phoenix 64 WINNONLIN software (version 6.3; Pharsight Certara, Palo Alto, CA). This was only used in the MA phase. In the no MA period, the dose from the dietary caffeine intake prior to administration of the caffeine study dose was unknown; therefore, the calculation of apparent clearance [CL/F = Dose/area under the curve $(AUC)_{0-\infty}$] using NCA could not be performed. The caffeine pharmacokinetic data from the 24-hour MA period were previously published using an NCA approach (Perera et al., 2011).

Population Pharmacokinetic Model. Caffeine and paraxanthine concentration-time data were simultaneously modeled in the present pharmacometric analysis. The observations obtained for the MA and no MA periods were fitted separately but were tested with the same structural pharmacokinetic models to avoid modeling it as interoccasion variability, which would add a further level of complexity to this model that was not deemed to be warranted. The pharmacokinetics of caffeine and paraxanthine were characterized by fitting candidate population pharmacokinetic models to the data, using a maximum likelihood estimation method available in ADAPT V (D'Argenio et al., 2009). The plasma concentrations of caffeine and paraxanthine collected 5 minutes prior to administration of the study dose were incorporated into each respective data set to ensure typical values for the first-order absorption rate constant (k_a) and absorption lag time (T_{Lag}). Therefore, the predose concentrations were given a time of 0, whereas the dose was administered at a time of 0.01. The initial conditions were estimated for all compartments (in milligrams). In the final model, the values for the peripheral compartment of caffeine and the absorptive compartment were fixed to avoid model overparameterization associated with estimating the amounts of caffeine in these compartments.

Residual Variance Models. Residual variance models were tested for both caffeine and paraxanthine. The empirical variance models assumed that the random errors in measurements of concentrations of caffeine and paraxanthine in plasma were similar for all study subjects and that the residual (error) S.D. of the observations (σ) was linearly related to the true values (Y): $\sigma = SD_{slope}\, Y + SD_{intercept}$, in which SD_{slope} and $SD_{intercept}$ are the variance parameters. The initial empirical estimates for the variance parameters were based on the assay performance. Later in the process, the values for $SD_{intercept}$ for both variance models of caffeine and paraxanthine were fixed to the LOQ of the assay while both slopes were estimated. The LOQ of the assay was 0.025 μg/ml for caffeine and paraxanthine.

Model Evaluation. Model discrimination was accomplished using the rule of parsimony (Jusko, 1992) and Akaike's information criterion (AIC) (Akaike, 1979). Table 2 presents the model parameters, their population mean estimates, the relative standard error as a percentage (RSE%) as a measure of the precision of the parameter estimates, and the coefficient of variation percentage as a measure of the true biologic variability between the participants, both for the MA and no MA periods. The AICs of the final model were −1045.52 and −565.67 for the MA and no MA periods, respectively. A number of models were evaluated, including a one-compartment pharmacokinetic model of caffeine (AICs of −953.32 and −332.32 in the MA and no MA periods, respectively) and a two-compartment pharmacokinetic model of paraxanthine (AICs of −896.60 and −286.61 in the MA and no MA periods). Other models evaluated with specific relevance to the research aim included addition of Michaelis-Menten (MM) kinetics as opposed to the first-order elimination describing pathways of caffeine or paraxanthine elimination and parallel first-order and MM kinetics to describe caffeine elimination. No improvement in the AIC was observed with the use of MM kinetics to describe the CL_{CAPX} (clearance of caffeine to paraxanthine) pathway (AICs of −1023.34 and −288.34 in the MA and no MA periods, respectively) or CL_{PX} (clearance of paraxanthine) (AICs of −855.45 and −300.43 in the MA and no MA periods, respectively).

Diagnostic plots, such as population predicted versus observed concentrations, individual predicted versus observed concentrations, and standardized weighted residuals versus time, were investigated to ensure that the chosen structural model accurately described the data. These plots were considered for both caffeine and paraxanthine in the MA and no MA periods. The simulation option in ADAPT V, was used to calculate the area under the curve of both paraxanthine and caffeine for 0–24 hours after the study dose (AUC_{0-24}). ADAPT V calculated the area under the curve by numerical integration of the fitted functions. To account for residual caffeine and paraxanthine concentrations in the no MA period, the initial conditions were set to 0 in each participant prior to simulating from the individual estimates of the pharmacokinetic parameters to calculate the AUC_{0-24} of caffeine and paraxanthine. The simulation option was also used to evaluate the predictive performance of the model by visual predictive checks. On the basis of the final model parameter estimates from each of the periods, 1000 virtual subjects were simulated in the MA and no MA periods. Visual predictive checks of the median of the simulated concentration-time curves against the observed median for caffeine and paraxanthine in the MA period with 90% prediction intervals were plotted using R software (R Development Core Team, 2013).

Handling Data Below the LOQ. Data below the LOQ were accommodated by the M3 method proposed by Beal (2001). Individual plasma concentrations, suspected to be outliers, were tested as follows. If the residual of the observation (the difference between fitted and observed values) was 3 or more S.D. of the measurement, and if the trajectory of the fitted line changed when the value was removed, the point was declared an outlier (Forrest et al., 1993). In total, six data points from the no MA period were deemed as outliers in the period, whereas no data were excluded from the MA period.

Statistical Analysis. Statistical analysis was conducted in SYSTAT v13.0 software (version 13.0; Systat Software, Inc., Chicago, IL). A related-groups t test was used to investigate differences between various pharmacokinetic parameters in the 24-hour MA and no MA phases. One-way analysis of variance was used to investigate differences among HCCs, MCCs, and LCCs. Pearson's correlation was used to investigate the correlation between pharmacokinetic parameters in either phase. Results are reported as the median and range or the mean ± S.D., depending on the distribution of the data. Where appropriate, a 95% confidence interval is also presented. All P values were reported as significant if $P < 0.05$.

Results

Final Pharmacokinetic Structural Model. The primary goal of modeling these data was to select a structural model to estimate the individual clearance parameters associated with the metabolism of caffeine. Caffeine and paraxanthine concentration-time data were simultaneously modeled. Figure 2 outlines the final pharmacokinetic model used in this analysis. Caffeine concentration-time data were best described by a two-compartment pharmacokinetic model with two linear clearance parameters: CL_{CAPX}, which describes the clearance of caffeine to paraxanthine, and CL_{CAO} (clearance of caffeine to other metabolites plus renal clearance), which describes clearance by all other elimination pathways of caffeine. Paraxanthine concentration-time data were described by a one-compartment

TABLE 2

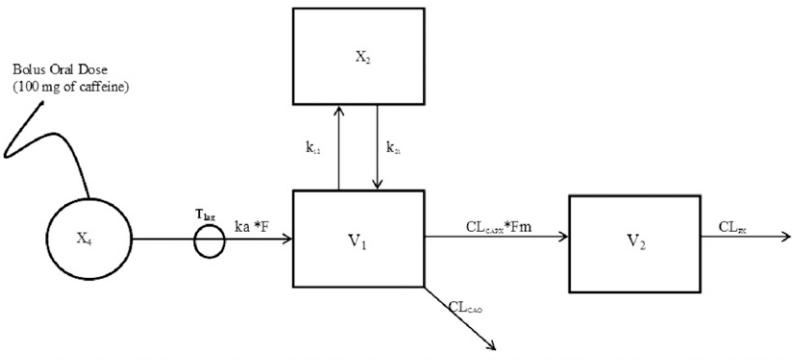
Model parameter estimates in the 24-hour MA period and no MA period

Parameter	24-Hour MA Period			No MA Period		
	Population Mean Estimate	RSE%	CV%	Population Mean Estimate	RSE%	CV%
k_a (h^{-1})	3.65	41.6	103	3.55	54.9	56.0
k_{12} (h^{-1})	1.71	19.4	32.5	1.54	71.5	62.6
k_{21} (h^{-1})	2.02	14.4	23.1	2.40	64.9	29.7
V_1 (liters)	31.2	38.8	17.7	27.7	56.8	20.2
V_2 (liters)	24.4	47.2	24.6	18.4	52.8	37.2
T_{Lag} (h^{-1})	0.15	54.5	107	0.14	76.2	81.2
CL_{CAPX} (l/h)	2.73	15.0	22.7	2.63	17.3	19.6
CL_{PX} (l/h)	3.81	11.2	13.4	3.70	14.2	10.9
CL_{CAO} (l/h)	4.86	40.1	57.1	3.74	49.0	68.1
IC(1) (mg)	1.72	82.3	229	3.40	144	187
IC(2) (mg)	1.20	Fixed	—	1.21	Fixed	—
IC(3) (mg)	4.06	22.8	48.2	6.93	77.2	103
IC(4) (mg)	1.37	Fixed	—	1.18	Fixed	—
$SD1_{intercept}$	0.05	Fixed	—	0.05	Fixed	—
$SD1_{slope}$	0.02	38.8	—	0.07	13.7	—
$SD2_{intercept}$	0.05	Fixed	—	0.05	Fixed	—
$SD2_{slope}$	0.04	32.2	—	0.04	29.9	—

CV%, coefficient of variation percentage (represents the true biologic variability between the participants); IC(i), Initial condition in the i^{th} compartment; RSE%, relative standard error as a percentage (represents the precision of the parameter estimate); $SD1_{intercept}$, variance model parameter describing additive residual error of caffeine; $SD1_{slope}$, variance model parameter describing the proportional residual error of caffeine; $SD2_{intercept}$, variance model parameter describing additive residual error of paraxanthine; $SD2_{slope}$, variance model parameter describing proportional residual error of paraxanthine.

pharmacokinetic model with one linear clearance term (CL_{PX}).The final model provided an excellent fit for the parent and metabolite concentration-time data in the 24-hour MA period [overall $r^2 = 0.987$ for caffeine (Fig. 3A) and $r^2 = 0.912$ for paraxanthine (Fig. 3B)] and no MA period [$r^2 = 0.983$ for caffeine (Fig. 3C) and $r^2 = 0.910$ for paraxanthine (Fig. 3D)]. In the MA period, the ranges of r^2 values for individual participant concentration-time data for caffeine and paraxanthine were 0.70–1.00 and 0.74–0.99, respectively. In the no MA period, the ranges of r^2 values were 0.90–1.00 and 0.70–0.99, respectively. Scatter diagnostic plots of observed versus individual predicted concentrations for both caffeine and paraxanthine in the MA and no MA periods showed that differences between pairs of observed and predicted values were small and normally distributed. Figure 4, A and B, shows the standardized weighted residuals over time for caffeine and paraxanthine, respectively, in the 24-hour MA phase. Similarly, Fig. 4, C and D, shows the standardized weighted residuals over time of caffeine and paraxanthine, respectively, in the no MA phase. Other diagnostic plots— including population predicted concentrations versus observed concentrations, which are not shown here—demonstrated good fits of the data for all subjects.

In this model, the drug is administered into an absorptive compartment with the amount of drug available systemically dependent on F, the oral bioavailability of the drug. After a lag time (T_{Lag}), caffeine is absorbed per a first-order absorption rate constant (k_a) into the central compartment (of apparent volume, V_1). Drug in the central compartment equilibrates via distributional rate constants (k_{12}, k_{21}) with drug in the peripheral compartment (X_2), and is eliminated from the central compartment by two linear clearance terms. One linear clearance eliminates to a separate compartment forming paraxanthine through a first-order process (CL_{CAPX}) and formation of other metabolites and renal elimination are considered together as a separate clearance parameter (CL_{CAO}). This grouping was necessary because there is limited information concerning the renal clearance of caffeine and formation of other metabolites. The apparent clearance of caffeine (CL) was calculated as the sum of CL_{CAPX} and CL_{CAO}. Paraxanthine was eliminated from this third compartment (with volume V_2) by linear



ka – rate constant from absorptive compartment; k_{12} – rate constant from compartment 1 of caffeine to peripheral compartment; k_{21} – rate constant from peripheral compartment to caffeine central compartment; V_1 – volume of distribution of caffeine in compartment 1; V_2 – volume of distribution of paraxanthine in compartment 3; ***Tlag*** – lag time from absorptive compartment; CL_{CAPX} – Clearance of caffeine to paraxanthine; CL_{PX} – Clearance of paraxanthine; CL_{CAO} – renal clearance of caffeine plus metabolic clearance of caffeine to theophylline and theobromine; F – bioavailability of caffeine (fixed at 1); ***Fm*** – Fraction of parent drug converted to metabolite (not estimated). X_2 – peripheral compartment; X_4 - absorptive compartment.

All distribution and elimination parameters of caffeine are predicated on F while all distribution and elimination parameters of paraxanthine are predicated on Fm.

Fig. 2. Population pharmacokinetic model of caffeine and paraxanthine.

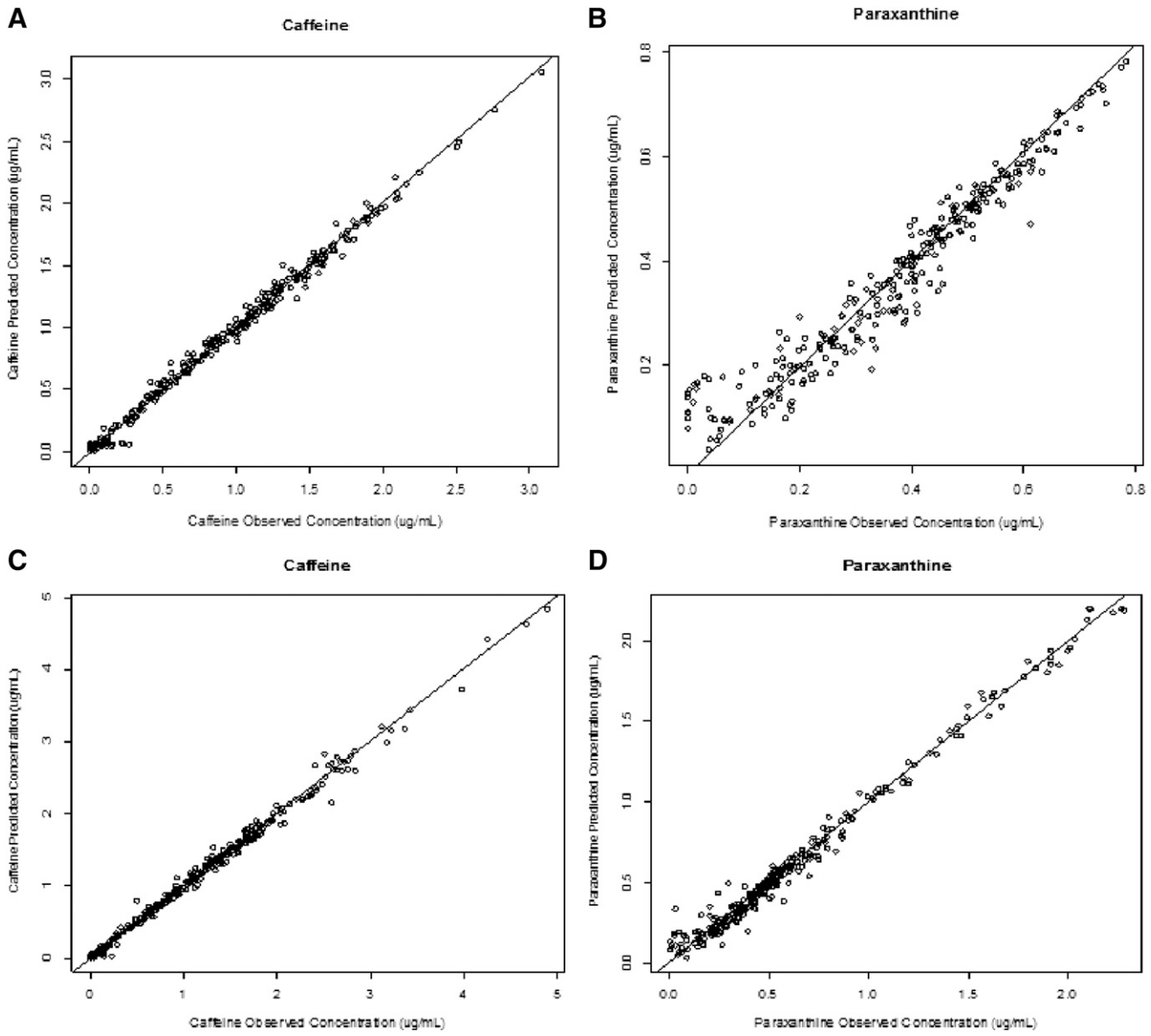


Fig. 3. Individual model-predicted versus observed concentrations (in micrograms per milliliter) for caffeine in the 24-hour MA phase (A), paraxanthine in the 24-hour MA phase (B), caffeine in the no MA phase (C), and paraxanthine in the no MA phase (D).

clearance (CL_{PX}). Because caffeine was administered as an oral dose, all of the fitted volumes and clearances are conditioned on *F* (i.e., apparent clearances and apparent volumes), which could not be estimated in a study of this design because of lack of plasma concentrations of caffeine after intravenous dosing. All distribution and elimination parameters of caffeine are predicated on *F*, whereas all distribution and elimination parameters of paraxanthine are predicated on *Fm* (the fraction of systemic caffeine that is metabolized to paraxanthine). Previous studies have indicated that there is complete bioavailability of oral caffeine; therefore, *F* was assumed to be 1 in all subjects (Blanchard and Sawers, 1983). Likewise, V_2 and CL_{PX} are also conditioned on *Fm* (the fraction of systemic caffeine which is metabolized to paraxanthine). Other pharmacokinetic parameter values were derived from the estimated parameters.

Figure 5, A and B, shows an example profile of the model-predicted caffeine and paraxanthine data, respectively, of an individual in the MA phase. Figure 5, C and D, shows the model-predicted caffeine and paraxanthine concentration-time profile, respectively, in the same individual in the no MA phase. Figure 6 shows the visual predictive checks for caffeine and paraxanthine in the MA phase. The graphs demonstrate that the model well predicts the observed concentrations, with approximately 10% of the simulated data lying outside the 90% prediction interval (shown in the shaded area). The visual predictive check is not shown for the no MA period because it is not designed to assess models containing large variability in the initial conditions (caffeine consumption).

Table 2 outlines the parameter estimates from the final population pharmacokinetic model. For the purposes of the current study, the clearance parameters related to caffeine (CL_{CAPX} and CL_{CAO}) and paraxanthine (CL_{PX}) elimination were of particular interest. The RSE%s (which indicate the precision of the parameter estimate) for CL_{CAPX}, CL_{CAO}, and CL_{PX} were 15.0, 11.2, and 40.1, respectively, in the MA period and 17.3, 14.2, and 49.0, respectively, in the no MA period. The RSE% and S.D. of the coefficient of variation percentage was high for the initial conditions when estimated; however, this was a reflection of the various different levels of caffeine intake in the

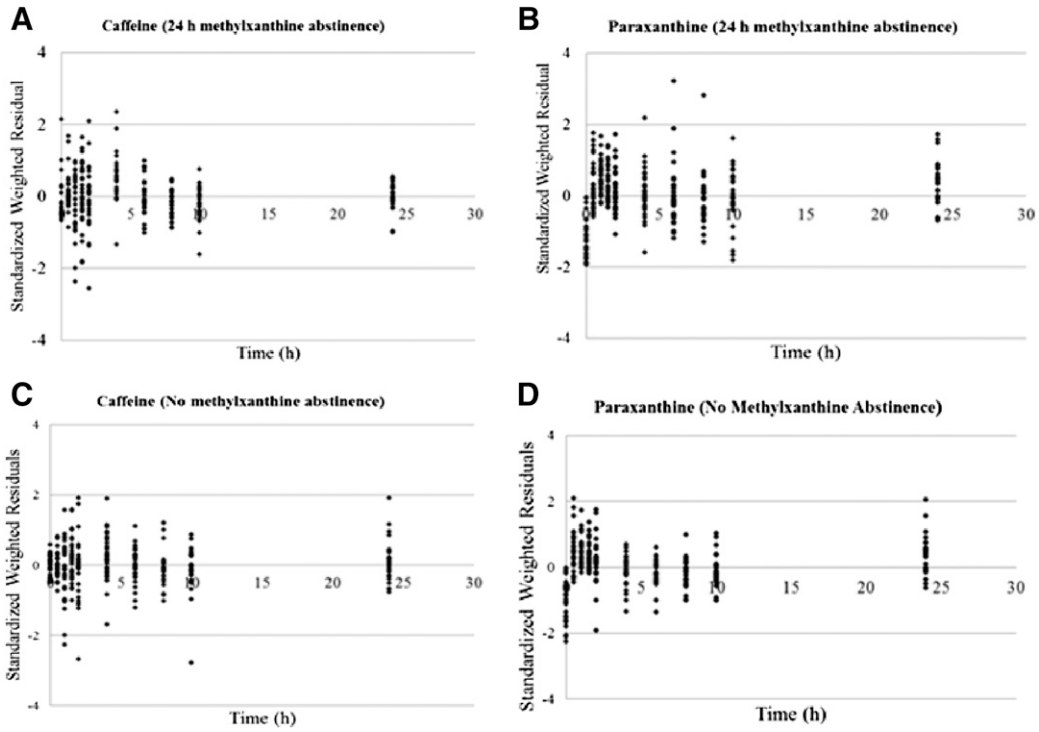


Fig. 4. Standardized weighted residuals versus time after dose (in hours) for caffeine in the 24-hour MA phase (A), paraxanthine in the 24-hour MA phase (B), caffeine in the no MA phase (C), and paraxanthine in the no MA phase (D).

population. The remaining parameters had excellent precision relative to the complexity of the final model providing confidence in the model-derived parameter estimates.

Concordance between Estimates of Total Apparent Caffeine Clearances. The concordance of the NCA and model-derived pharmacokinetic parameter estimates for concentration-time data generated in the

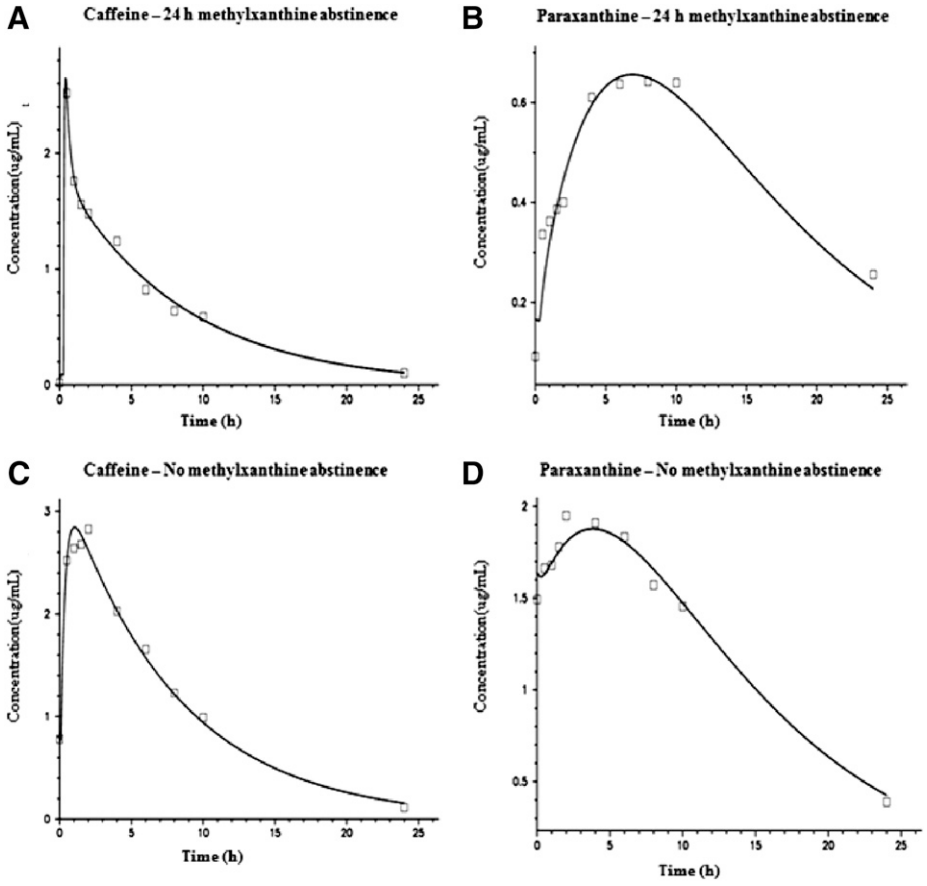


Fig. 5. Predicted caffeine and paraxanthine concentrations in a representative participant in the 24-hour MA period (A and B) and the no MA period (C and D), respectively.

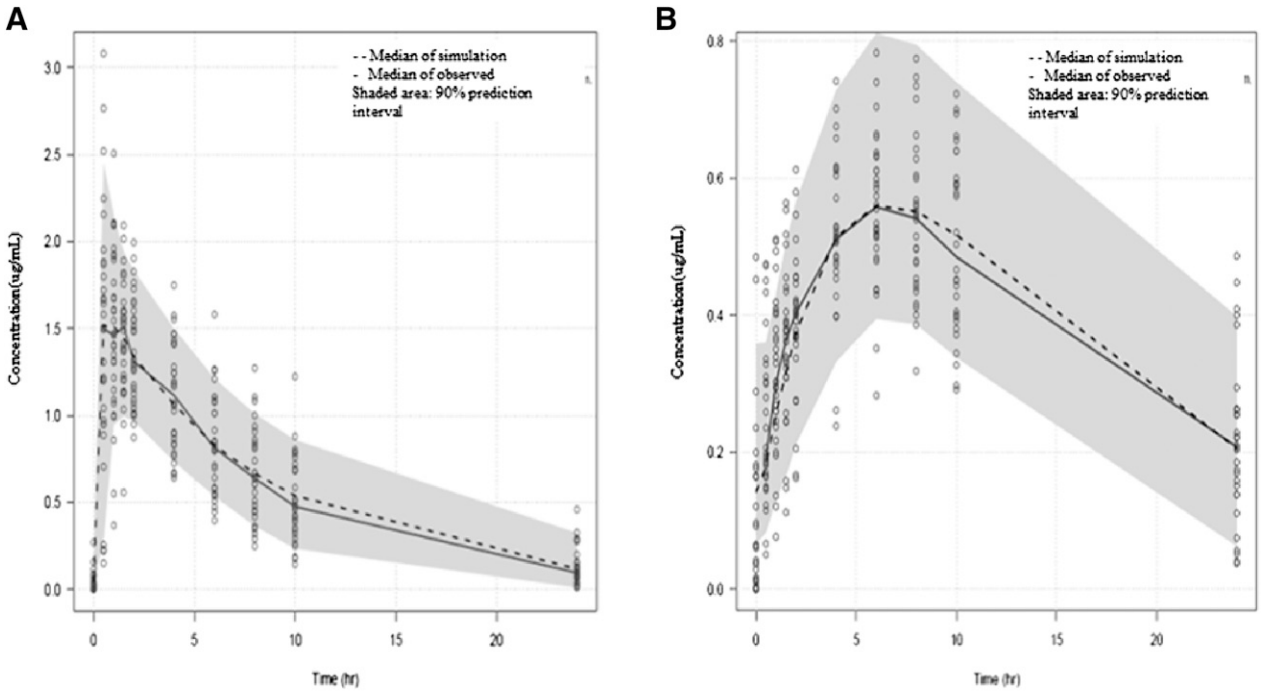


Fig. 6. Visual predictive check for caffeine (A) and paraxanthine (B) in the 24-hour MA period.

MA period was excellent (regression line not different from line of identity, $r^2 = 0.994$). Model-derived estimates for clearance in the no MA period and either NCA or model-derived parameter estimates in the MA period were systematically different and were less strongly correlated ($r^2 = 0.72$). In the 24-hour MA period, the total apparent caffeine clearance was significantly higher (8.44 ± 3.30 l/h) compared with the clearance in the no MA period (7.24 ± 3.03 l/h) ($P < 0.05$).

Impact of Caffeine Consumption. The average predose caffeine and paraxanthine concentrations in the 30 participants after the 24-hour MA period were 0.04 and 0.10 μg/ml, respectively. In the no MA period, the predose caffeine and paraxanthine concentrations were both 0.40 μg/ml. Among the LCCs or MCCs, no significant difference was observed between the MA period and no MA period in the clearances of caffeine (CL_{CAPX}, CL_{CAO}), paraxanthine (CL_{PX}), or total apparent caffeine clearance (CL). In participants characterized as HCCs, a significant difference was observed between the MA period and no MA period in the CL_{CAO} (clearance of caffeine by renal elimination and/or formation of other metabolites) and CL ($P < 0.05$) but not in CL_{CAPX} or CL_{PX} (Table 3). In the MA period, a significant difference in CL_{CAO} was detected between the HCCs ($n = 5$; 7.51 ± 3.36 l/h) and LCCs ($n = 16$; 4.28 ± 1.93 l/h) ($P < 0.05$) and the MCCs ($n = 9$; 7.05 ± 3.11 l/h) and LCCs ($n = 16$; 4.28 ± 1.93 l/h) ($P < 0.05$). Similarly, in the no MA period, there was a significant difference between the MCCs ($n = 9$; 6.77 ± 3.07 l/h) and HCCs ($n = 5$; 3.59 ± 2.16 l/h) ($P < 0.05$) and the MCCs ($n = 9$; 6.77 ± 3.07 l/h) and LCCs ($n = 16$; 3.67 ± 2.08 l/h) ($P < 0.05$). There was no significant difference between high, medium or low caffeine consumers in the other clearance parameters (CL_{CAPX} or CL_{PX}) in the MA or no MA period ($P > 0.05$).

Table 4 outlines the AUC_{0-24} of caffeine and paraxanthine stratified by low, medium, or high caffeine consumption in the MA and no MA periods. The only observed difference was in the AUC_{0-24} of caffeine among high caffeine consumers between the two periods ($P < 0.05$). In agreement with the original study, the paraxanthine/caffeine AUC_{0-24} metabolic ratio was not significantly different among HCCs or LCCs in either period ($P > 0.05$). Among HCCs, the paraxanthine/caffeine AUC_{0-24} metabolic ratio decreased between the two periods compared with the CL_{PX}/CL ratio, which increased (Tables 3 and 4) ($P > 0.05$). The correlations between the paraxanthine/caffeine AUC_{0-24} metabolic ratio and the CL_{CAPX} in the MA and no MA periods were 0.89 and 0.98, respectively.

Discussion

This research has developed a population pharmacokinetic model that simultaneously describes caffeine and paraxanthine pharmacokinetics to investigate the impact of varying levels of caffeine consumption on pathways associated with caffeine and paraxanthine elimination. The modeling indicated there was a significantly lower total apparent caffeine clearance in the no MA phase compared with a 24-hour MA phase. When stratified by high, medium, or low caffeine consumers (> 200 mg/d, 100–200 mg/d, or <100 mg/d, respectively), this difference was only present among HCCs. The decrease in the total apparent caffeine clearance in the no MA period does not appear related to saturation of the CYP1A2 enzyme pathway, as indicated by the lack of change in the clearance of caffeine to paraxanthine and the clearance of paraxanthine, both of which are primarily mediated by CYP1A2.

Caffeine has a complex metabolism (Perera et al., 2012b). The hepatic N-3 demethylation of caffeine to form paraxanthine is reported to be exclusively mediated by CYP1A2 (Butler et al., 1992; Gu et al., 1992), whereas formation of other metabolites is due to multiple enzymes, including CYP2E1 and CYP3A4 (Berthou et al., 1991; Tassaneeyakul et al., 1994). It has also been suggested that at higher caffeine concentrations, enzymes other than CYP1A2 play a role in its metabolism (Birkett et al., 1985; Gu et al., 1992). The model in this study indicated that in HCCs, MCCs, and LCCs, the clearance of caffeine to paraxanthine (CL_{CAPX}) and the clearance of paraxanthine (CL_{PX}) did not change between the 24-hour MA period and the no MA period; however, the total apparent caffeine clearance (CL),

TABLE 3

Population clearance parameters of caffeine and paraxanthine in LCCs, MCCs, and HCCs

The *P* values reflect paired samples *t* test between the two periods of the same metrics. All clearances are expressed in l/h.

Consumption Group	24-Hour MA		No MA		Significance
	Mean ± S.D.	95% CI	Mean ± S.D.	95% CI	
LCCs (n = 16) (0–100 mg/d)					
CL	6.88 ± 2.40	5.61–8.16	6.22 ± 2.33	4.97–7.46	NS
CL_{CAPX}	2.61 ± 0.55	2.31–2.90	2.55 ± 0.45	2.31–2.79	NS
CL_{CAO}	4.28 ± 1.93	3.25–5.31	3.67 ± 2.08	2.56–4.78	NS
CL_{PX}	3.77 ± 0.30	3.61–3.93	3.70 ± 0.18	3.60–3.80	NS
CL_{PX}/CL	0.61 ± 0.21	0.50–0.72	0.68 ± 0.26	0.54–0.81	NS
MCCs (n = 9) (100–200 mg/d)					
CL	10.09 ± 3.27	7.57–12.60	9.68 ± 3.30	7.12–12.24	NS
CL_{CAPX}	3.04 ± 0.47	2.68–3.40	2.91 ± 0.39	2.60–3.21	NS
CL_{CAO}	7.04 ± 3.11	4.65–9.44	6.77 ± 3.07	4.41–9.13	NS
CL_{PX}	3.98 ± 0.28	3.77–4.19	3.73 ± 0.09	3.66–3.79	NS
CL_{PX}/CL	0.44 ± 0.17	0.31–0.57	0.43 ± 0.15	0.31–0.54	NS
HCCs (n = 5) (>200 mg/d)					
CL	10.47 ± 3.91	5.62–15.33	6.30 ± 2.34	3.40–9.20	<0.05
CL_{CAPX}	2.97 ± 0.59	2.24–3.70	2.71 ± 0.31	2.31–3.10	NS
CL_{CAO}	7.51 ± 3.36	3.35–11.67	3.59 ± 2.16	0.91–6.27	<0.05
CL_{PX}	3.86 ± 0.07	3.77–3.96	3.78 ± 0.26	3.46–4.10	NS
CL_{PX}/CL	0.42 ± 0.19	0.19–0.65	0.68 ± 0.31	0.30–1.06	<0.05

CI, confidence interval; CL, total clearance of caffeine; CL_{CAO}, clearance of caffeine to other metabolites plus renal clearance; CL_{CAPX}, clearance of caffeine to paraxanthine; CL_{PX}/CL, fraction metabolized by CYP1A2 pathway; NS, not signficiant.

which is a sum of all pathways of caffeine elimination, was significantly lower in the no MA period among HCCs only. This indicates that the activity of CYP1A2 does not differ between the two periods and that the observed change in the apparent caffeine clearance is due to a change in renal clearance of caffeine or formation of other metabolites of caffeine.

Generally, nonlinear pharmacokinetics that results in a decrease in total apparent clearance is due to either saturable metabolism (e.g., phenytoin) and/or saturable renal elimination (e.g., piperacillin) (Jusko et al., 1976; Bulitta et al., 2010). Numerous studies have been conducted to establish the linear concentration range of caffeine in humans; however, conflicting results have been reported (Newton et al., 1981; Bonati et al., 1982; Cheng et al., 1990; Denaro et al., 1990; Hetzler et al., 1990; Nickell and Uhde, 1994; Lee et al., 1997; Doude van Troostwijk et al., 2003). Bonati et al. (1982) described caffeine pharmacokinetics to be linear up to a dose of 10 mg/kg, whereas Newton et al. (1981) also suggested linearity between single doses of 50 and 750 mg in humans. In the present study, the highest consumption of caffeine among all participants was 340 mg/d; on the basis of the previously mentioned studies, the addition of 100 mg of caffeine is unlikely to result in a dose-dependent change in its pharmacokinetics.

Previous studies have indicated that the renal clearance of caffeine is negligible relative to its hepatic metabolism (Birkett and Miners, 1991). However, the relative balance of filtration, secretion, and reabsorption determines the kinetics of drug elimination by the kidneys. With high urine flow, less of the drug may be reabsorbed and more of the drug is excreted, whereas with low urine flow, more of the drug is reabsorbed leading to less drug excretion (Hacker et al., 2009). Increased urine flow has been shown to increase renal caffeine clearance (Trang et al., 1985; Birkett and Miners, 1991). In individuals who regularly consume caffeine, a tolerance to its diuretic effect is well known and can develop over a period of 1–5 days of regular caffeine use (Maughan and Griffin, 2003). However, many of the pharmacodynamic effects, including blood pressure and norepinephrine concentrations, show acute sensitivity to a caffeine dose after caffeine abstinence (Denaro et al., 1991). Studies have shown that acute ingestion of caffeine (250–300 mg) after an abstinence of

TABLE 4

AUC values from population model stratified by high, medium, or low caffeine consumption and abstinence period

The *P* values reflect paired samples *t* test between the two periods of the same metrics. The units of AUC are expressed as μg/ml·h.

AUC_{0-24}	24-Hour MA		No MA		Significance
	Mean ± S.D.	95% CI	Mean ± S.D.	95% CI	
LCCs (n = 16)					
Caffeine	15.17 ± 4.45	12.80–17.55	17.11 ± 6.78	13.50–20.72	NS
Paraxanthine	9.92 ± 2.35	8.67–11.17	10.15 ± 3.68	8.19–12.11	NS
Paraxanthine/caffeine (AUC metabolic ratio)	0.60 ± 0.15	0.60–0.76	0.61 ± 0.12	0.54–0.68	NS
MCCs (n = 9)					
Caffeine	10.96 ± 4.03	7.86–14.06	10.74 ± 3.26	8.24–13.25	NS
Paraxanthine	7.60 ± 2.41	5.74–9.55	7.20 ± 1.34	6.18–8.24	NS
Paraxanthine/caffeine (AUC metabolic ratio)	0.71 ± 0.12	0.62–0.80	0.70 ± 0.13	0.60–0.79	NS
HCCs (n = 5)					
Caffeine	10.75 ± 4.45	5.22–16.27	16.87 ± 6.37	8.96–24.78	<0.05
Paraxanthine	7.52 ± 1.64	5.49–9.56	10.24 ± 2.59	7.02–13.45	NS
Paraxanthine/caffeine (AUC metabolic ratio)	0.75 ± 0.16	0.55–0.94	0.63 ± 0.10	0.50–0.76	NS

CI, confidence interval; NS, not significant.

caffeine for a period of days or weeks results in a short-term stimulation of urine output (Nawrot et al., 2003). It is hypothesized that there is increased sensitivity to the diuretic effect of caffeine in our study, resulting in an increased apparent caffeine clearance in HCCs after a 24-hour MA period. In the no MA period, this sensitivity is not present due to the tolerance associated with regular caffeine consumption and thus the overall apparent caffeine clearance is lower.

The other option that must be considered is the potential change to formation and elimination of other metabolites, which may change the overall caffeine clearance. A study by Kaplan et al. (1997) reported a dose-dependent effect of caffeine with a significantly lower clearance of caffeine observed after a 500 mg oral dose of caffeine compared with a 250 mg dose of caffeine. The researchers found that there was no difference between the paraxanthine $AUC_{0–8h}$ or theophylline $AUC_{0\text{-}8h}$. However, a difference was observed for the $AUC_{0–8h}$ of theobromine between the two doses, indicating a nonlinear pathway associated with formation of theobromine after caffeine consumption. This result is indicative of a change in a pathway other than CYP1A2. Berthou et al. (1995b) reported that there was a significant interaction between two probe drugs, caffeine and chlorzoxane, which were used to measure CYP1A2 and CYP2E1 activity, respectively. In this study, a 140 mg dose of caffeine was given together with chlorzoxazone and a 20% decrease in the plasma caffeine metabolic ratio (paraxanthine/caffeine) was observed. The authors hypothesized that this was due to competitive inhibition, as demonstrated by in vitro data. Similarly, Tang-Liu et al. (1983) reported that at least one and possibly more routes of elimination of caffeine may be saturable and/or its metabolites may inhibit xanthine oxidase. These results raise the possibility that the CYP2E1 pathway plays a larger role in humans in vivo in alterations in the caffeine metabolic ratios than previously thought.

With respect to measurement of CYP1A2 activity, the modeling results demonstrated that the correlation between the CL_{CAPX} and the paraxanthine/caffeine $AUC_{0–24}$ metabolic ratio was greater in the no MA period compared with the 24-hour MA period. This indicates that paraxanthine/caffeine ratios obtained in the no MA period provide better estimates of CYP1A2 activity and that variability associated with paraxanthine/caffeine ratios is likely due to the forced 24-hour abstinence from caffeine.

There are limitations of this study primarily associated with the model used. The final pharmacokinetic model used in this study is nonidentifiable in one respect because only caffeine and paraxanthine concentrations were available in plasma and no data were available on urine concentrations of metabolites or unchanged caffeine. Therefore, the pathway of caffeine leading to the formation of other metabolites and renal clearance of caffeine is speculative. However, the model used in this study was considered the best option given the available data to generate hypotheses for the changes in pharmacokinetics of caffeine that were observed. Furthermore, there would be limitations surrounding the parameters of an identifiable model including the following: a single clearance pathway of caffeine leading to paraxanthine would assume that 100% of caffeine is converted to paraxanthine, and the volume of distribution of paraxanthine would be conditioned on the total caffeine clearance rather than the clearance of caffeine to paraxanthine. Our results could also indicate a change in the bioavailability of caffeine between the two phases of the study. However, previous studies demonstrated that when comparing intravenous and oral doses, the absolute bioavailability of caffeine has been reported as 100% with varying oral doses and is not concentration dependent (Blanchard and Sawers, 1983). There are numerous advantages to this modeling approach. The identification of a single pathway related to the CYP1A2 enzyme pathway (CL_{CAPX}) can be considered a gold standard measure of CYP1A2 activity, eliminating the influence of other enzyme pathways that are reflected in the total apparent clearance or metabolic ratios. This model, which was developed based on rich pharmacokinetic data, can be used with Bayesian approaches to derive accurate results for other data sets that may include only sparse data for caffeine and paraxanthine. The pharmacokinetic model for caffeine may also have applications in disease states such as liver disease, for which the typical fasting period is not adequate due to the prolonged half-life of caffeine.

The model developed in this study, which simultaneously described caffeine and paraxanthine concentration-time data, indicated that a MA period has a significant impact on the pharmacokinetics of caffeine in HCCs. These results have several implications for the utilization of caffeine metrics to investigate CYP1A2 phenotype-genotype associations and dose individualization of CYP1A2 metabolized medicines. Future studies are warranted to investigate the impact of varying MA periods on the pharmacokinetics of caffeine and its metabolites in HCCs and to measure the concentrations of caffeine and metabolites in both plasma and urine.

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Authorship Contributions

Participated in research design: Perera, Gross, Xu, McLachlan.
Conducted experiments: Perera, Xu, McLachlan.
Contributed new reagents or analytic tools: McLachlan.
Performed data analysis: Perera, Forrest, Landersdorfer.
Wrote or contributed to the writing of the manuscript: Perera, Forrest, Landersdorfer, Gross, Ait-Oudhia, McLachlan.

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