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

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Abstract

Aim: The aim of this study was to investigate the impact of methylxanthine abstinence 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. Methods: 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 methylxanthine abstinence (MA) period. Participants were classified as low (LCC: 0 – 100 mg/day), medium (MCC: 100 – 200 mg/day) or high (HCC: >200 mg/day) caffeine consumers. All caffeine and paraxanthine concentration-time data were simultaneously modelled. Caffeine pharmacokinetics were 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. Results: Among LCC (n=16) and MCC (n = 9) there was no difference in the average total apparent caffeine clearance (CL) between the MA (mean, 95% CI) (LCC: 6.88, 5.61 – 8.16 L/h), (MCC: 10.09, 7.57 – 12.60 L/h) and the no MA periods (LCC: 6.22, 4.97 – 7.46L/h), (MCC: 9.68, 7.12 - 12.24L/h). The CL among HCC (n=5) was considerably higher in the MA (10.48, 5.62 – 15.33 L/h) compared to the no MA (6.30, 3.40 – 9.20 L/h) period (p<0.05). Conclusions: The decrease in CL in the no MA period amongst HCC appears to be due to alternative caffeine elimination pathways rather than CYP1A2.
Introduction

The cytochrome P450 1A2 (CYP1A2) enzyme contributes to the metabolism of approximately 11% of all therapeutically used medicines including olanzapine, clozapine and theophylline (Faber et al., 2005; Perera, Gross, T.M., et al., 2013). CYP1A2 activity demonstrates a large degree of variability in human populations with a 10-15 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; Djordjevic et al., 2008; Ghotbi et al., 2007; Perera et al., 2012a; Perera, Gross, & McLachlan, 2013). There is also increasing interest in the use of CYP1A2 phenotype to individualize the dose of drugs metabolised by this enzyme pathway and therefore it is essential to ensure an accurate estimate of CYP1A2 activity is obtained (Perera, Gross, T.M., et al., 2013).

Currently, caffeine (1,3,7-trimethylxanthine) is 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 the total caffeine metabolism (Figure 1) (Gu et al., 1992; Kalow & Tang, 1993). The pathway forming paraxanthine is exclusively mediated by CYP1A2 while 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 co-administration of CYP1A2 inhibitors (e.g. furafylline) (Sesardic et al., 1990; Tarrus et al., 1987).
Measuring apparent caffeine clearance is considered to be a gold standard measurement of 
CYP1A2 activity (Kalow & Tang, 1993). However, based on the exclusive metabolism of 
caffeine to paraxanthine by CYP1A2, the ideal measurement would be the apparent clearance of 
caffeine to paraxanthine (Tucker et al., 1998). Typically, this is not a feasible measurement when 
non-compartmental pharmacokinetic methods are used unless paraxanthine is directly 
administered. Pharmacometric tools allow modelling of concentration-time data using prior 
knowledge of the pathways associated with metabolism and therefore can be used to generate 
estimates of clearance not usually obtained experimentally (Bulitta et al., 2010; Rostami-
Hodjegan et al., 1996).

Previous studies have indicated that at high or repeated doses, caffeine displays non-linear (dose-
dependent) pharmacokinetics (Blanchard & Sawers, 1983; Bonati et al., 1982; Cheng et al., 
1990; Denaro et al., 1991; Denaro et al., 1990; Renner et al., 1984). As 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., 1995; Perera et al., 2012a). Many *in vivo* studies have applied a methylxanthine 
abstinence period prior to CYP1A2 phenotyping in order to ensure complete washout of caffeine 
to conduct non-compartmental analysis (NCA) (Aklillu et al., 2003; Djordjevic et al., 2008; 
Ghotbi et al., 2007; Tantcheva-Poor et al., 1999). However, these studies have not considered the 
potential impact of methylxanthine abstinence 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 pharmacokinetics of caffeine and the impact of a methylxanthine abstinence period is an important consideration to ensure 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 methylxanthine abstinence 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 which investigated CYP1A2 metrics in plasma and saliva in thirty subjects (Perera et al., 2011). The previous study utilised NCA to calculate pharmacokinetic (PK) parameters, namely apparent caffeine clearance, in the MA period, however, was unable to obtain PK parameters in the no MA period due to the assumptions associated with NCA. Pharmacometric modelling allows the calculation of these PK parameters without the assumptions of NCA.

Study participants were healthy non-smoking male volunteers of South Asian or European ancestry who had an average (± SD) age of 24.0 ± 4.7 years, weight of 78.5 ± 11.4 kg and BMI of 24.5 ± 2.8 kg/m². Each participant received a 100 mg caffeine tablet (NoDoz Tablets, Key Pharmaceutical, North Ryde, NSW, Australia) on 2 occasions. In the first phase of the study, participants were asked to abstain from any methylxanthine containing foods and beverages for 24 h prior to ingestion of the caffeine study dose, while 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 – 10 h of the study. They were not observed during 10 – 24 h and therefore it is possible that the participants would have consumed caffeine in this period. However, we found no deviations in the 24 h concentration range that was outside the range of the typical values (based on more than 3 standard deviations 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 min prior to administration of the study dose and at 0.5, 1, 1.5, 2, 4, 6, 8, 10 and 24 h post-study dose. Following centrifugation, plasma was harvested and stored at -20ºC. Caffeine and paraxanthine concentrations were quantified following liquid-liquid extraction and a selective validated HPLC assay (Perera et al., 2010). The intraday and interday coefficients of variation of the assay were less than 15% while 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 (LOD) was 0.01 µg/mL for both analytes (Perera et al., 2011).

**Data Collection and Caffeine Intake**

Information regarding each participant’s diet, specifically relating to methylxanthine including caffeine intake was collected. 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 and participants were classified as ‘low (LCC)’, ‘medium (MCC)’ and ‘high (HCC)’ caffeine consumers ‘high if they had a caffeine intake between 0 – 100, 100 – 200 and >200 mg/d, respectively (Perera et al., 2012a). Sixteen participants reported caffeine intake less than 100 mg/d (LCC) (range; 0 – 75 mg/d mg/day), 9 reported caffeine intake between 100 and 200 mg/day (range; 110 – 155 mg/d) lowwhile 5 subjects were considered high caffeine consumers (range; 205 – 340 mg/d) (Table 1).

**Non-Compartmental Pharmacokinetic Methods**
The apparent caffeine clearance in the 24 h methylxanthine abstinence phase for each of the study participants was analysed by non-compartmental analysis (NCA) using Phoenix 64 WINNONLIN (version 6.3; Pharsight Certara, Palo Alto, CA, USA). This was only utilized 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 and therefore the calculation of apparent clearance (CL/F = Dose/AUC₀-∞) using non-compartmental analysis could not be performed. The caffeine pharmacokinetic data from the 24 h methylxanthine abstinence period has been previously published using an NCA approach (Perera et al., 2011).

**Population Pharmacokinetic Model**

Caffeine and paraxanthine concentration-time data were simultaneously modelled in the present pharmacometric analysis. The observations obtained for the MA period and no MA period were fitted separately but were tested with the same structural pharmacokinetic models in order to avoid modelling it as inter-occasion variability which would add a further level of complexity to this model which was not deemed to be warranted. The PK of caffeine and paraxanthine were characterized by fitting candidate population PK (pop PK) models to the data, using a maximum likelihood estimation method (MLEM) available in ADAPT V (D'Argenio et al., 2009). The plasma concentrations of caffeine and paraxanthine collected five 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 (kₐ) and absorption lag time (Tₐₙ). Therefore, the pre-dose concentrations were given a time of 0 while the dose was administered at a time of 0.01. The initial conditions were estimated for all compartments (in mg amount). In the final model the values for the peripheral compartment of caffeine and the absorptive compartment were fixed to
avoid model over-parameterization 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 of the subjects in the study and that the residual (error) SDs of the observations ($\sigma$) were linearly related to the true values ($Y$): $\sigma = SD_{\text{slope}} Y + SD_{\text{intercept}}$, in which $SD_{\text{slope}}$ and $SD_{\text{intercept}}$ 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 $SD_{\text{intercept}}$ for both variance models of caffeine and paraxanthine were fixed to the limits of quantification (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 deviation percentage (RSE %) as a measure of the precision of the parameter estimates and coefficient of variation percentage (CV%) as a measure of the true biological variability between the participants, both for the MA and no MA period. The AIC of the final model was -1045.52 and -565.67 for the MA and no MA, respectively. A number of models were evaluated including a one compartment pharmacokinetic model of caffeine ($AIC = -953.32$ in the MA and $AIC = -332.32$ in the no MA) and a two
compartment pharmacokinetic model of paraxanthine (AIC = -896.60 in the MA and AIC = -286.61 in the no MA). 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 $CL_{CAPX}$ pathway (AIC = -1023.34 in the MA and AIC = -288.34 in the no MA) or $CL_{PX}$ (AIC = -855.45 in the MA and AIC = -300.43 in the no MA).

Diagnostic plots such as population predicted vs. observed concentrations, individual predicted vs. observed concentrations and standardized weighted residuals vs. time were investigated to ensure that the structural model chosen accurately described the data. These plots were considered for both caffeine and paraxanthine in the MA and no MA period. The simulation option in ADAPT V, was used to calculate the area under the curve of both paraxanthine and caffeine for 0 – 24 h after the study dose ($AUC_{0-24}$). ADAPT V calculated the area under the curve by numerical integration of the fitted functions. In order 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 utilised to evaluate the predictive performance of the model by visual predictive checks. Based on 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 with 90% prediction intervals were plotted using R (Team, 2013).

Handling Data Below the Limit of Quantification
Data Below the Limit of Quantification (BLQ) were accommodated by the M3 method proposed by Beal (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 standard deviations (SDs) 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 6 data points from the no MA period were deemed as outliers in the period while no data were excluded from the MA period.

**Statistical Analysis**

Statistical analysis was conducted in SYSTAT v13.0. Related groups t-test was utilised to investigate differences between various pharmacokinetic parameters in the 24 h methylxanthine abstinence phase and no methylxanthine abstinence phase. One way analysis of variance (ANOVA) was utilised to investigate differences among HCC, MCC and LCChighlow. Pearson’s correlation was used to investigate the correlation between pharmacokinetic parameters in either phase. Results are reported as median and range or mean ± SD depending on the distribution of the data. Where appropriate a 95% CI is also presented. All p-values were reported as significant if p < 0.05.
Results

Final PK Structural Model

The primary goal of modelling 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 modelled. Figure 2 outlines the final pharmacokinetic model used in this analysis. Caffeine concentration-time data was 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} which describes clearance by all other elimination pathways of caffeine), while paraxanthine concentration-time data was described by a one compartment 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 h methylxanthine abstinence period (overall $r^2 = 0.987$ for caffeine, Figure 3A, and $r^2 = 0.912$ for paraxanthine, Figure 3B) and no MA period ($r^2 = 0.983$, Figure 3C, for caffeine, and $r^2 = 0.910$ for paraxanthine, Figure 3D). In the MA period, the range of the $r^2$ for individual participant concentration-time data for caffeine and paraxanthine was $0.70 – 1.00$ and $0.74 – 0.99$, respectively, while in the no MA period, the range of $r^2$ was $0.90 – 1.00$ and $0.70 – 0.99$, respectively. Scatter diagnostic plots of observed vs. individual predicted concentrations for both caffeine and paraxanthine in the MA period and no MA period showed that differences between pairs of observed and predicted values were small and normally distributed. Figure 4A and 4B show the standardized weighted residuals over time for caffeine and paraxanthine, respectively, in the 24 h methylxanthine abstinence phase. Similarly, Figure 4C and 4D show the standardized weighted residuals over time of caffeine and paraxanthine, respectively, in the no
MA phase. Other diagnostic plots, including population predicted concentrations vs. observed concentrations, which are not shown here, demonstrated good fits of the data for all subjects.

In this model, 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 ($T_{\text{Lag}}$), 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 ($CL_{\text{CAPX}}$) and formation of other metabolites and renal elimination are considered together as a separate clearance parameter ($CL_{\text{CAO}}$). This grouping was necessary as 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 $CL_{\text{CAPX}}$ and $CL_{\text{CAO}}$. Paraxanthine was eliminated from this third compartment (with volume $V_2$) by linear clearance ($CL_{\text{PX}}$). 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 $F$ while all distribution and elimination parameters of paraxanthine are predicated on $F_m$. Previous studies have indicated that there is complete bioavailability of oral caffeine and therefore $F$ was assumed to be 1 in all subjects (Blanchard & Sawers, 1983). Likewise, the $V_2$ and $CL_{\text{PX}}$ are also conditioned on $F_m$ (the fraction of systemic caffeine which is metabolised to paraxanthine). Other pharmacokinetic parameter values were derived from the estimated parameters.
Figure 5A and 5B show an example profile of the model predicted caffeine and paraxanthine data, respectively, of an individual in the MA phase. Figure 5C and 5D shows the model predicted caffeine and paraxanthine concentration-time profile, respectively, in the same individual in the no MA phase. Figure 6A and 6B show the visual predictive checks (VPC) for caffeine and paraxanthine in the MA phase. The graphs demonstrate that the model predicts the observed concentrations well, with approximately 10% of the simulated data lying outside the 90% prediction interval (shown in the shaded area). The VPC is not shown for the no MA period as 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 PK model. For the purposes of the current study, the clearance parameters related to caffeine (CL\textsubscript{CAPX} and CL\textsubscript{CAO}) and paraxanthine (CL\textsubscript{PX}) elimination were of particular interest. The RSE\% (which indicates the precision of the parameter estimate) for CL\textsubscript{CAPX}, CL\textsubscript{CAO} and CL\textsubscript{PX}, was 15.0, 11.2 and 40.1, respectively, in the MA period. In the no MA period the RSE\% for CL\textsubscript{CAPX}, CL\textsubscript{CAO} and CL\textsubscript{PX} was 17.3, 14.2 and 49.0, respectively. The RSE\% and standard deviation of the coefficient of variation (CV) percentage was high for the initial conditions when estimated, however this was a reflection of the various different levels of caffeine intake in the 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 abstinence 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 h MA period, the total apparent caffeine clearance (CL) was significantly higher \( (8.44 \pm 3.30 \text{ L/h}) \) compared to the CL in the no MA period \( (7.24 \pm 3.03 \text{ L/h}) \) \( (p < 0.05) \).

**Impact of Caffeine Consumption**

The average pre-dose caffeine and paraxanthine concentrations in the 30 participants following the 24 MA period were 0.04 and 0.10 µg/mL, respectively. In the no MA period, the pre-dose caffeine and paraxanthine concentrations were both 0.40 µg/mL. Among the low or medium caffeine consumers, no significant difference was observed between the MA period and no MA period in the clearances of caffeine \( (\text{CL}_{\text{CAPX}}, \text{CL}_{\text{CAO}}) \), paraxanthine \( (\text{CL}_{\text{PX}}) \) or total apparent caffeine clearance \( (\text{CL}) \). In participants characterised as high caffeine consumers, a significant difference was observed between the MA period and no MA period in the \( \text{CL}_{\text{CAO}} \) (clearance of caffeine by renal elimination and/or formation of other metabolites) and \( \text{CL} \) \( (p < 0.05) \) but not in \( \text{CL}_{\text{CAPX}} \) or \( \text{CL}_{\text{PX}} \) (Table 3). A significant difference in \( \text{CL}_{\text{CAO}} \) was detected between the high and low \( (n = 5, 7.51 \pm 3.36 \text{ vs } n = 16, 4.28 \pm 1.93, \text{ L/h respectively}) \) \( (p < 0.05) \) and medium and low \( (n = 9, 7.05 \pm 3.11 \text{ vs } n = 16, 4.28 \pm 1.93, \text{ L/h respectively}) \) \( (p < 0.05) \) caffeine consumers in the MA period. Similarly, in the no MA period, there was a significant difference between the medium and high \( (n = 9, 6.77 \pm 3.07 \text{ vs } n = 5, 3.59 \pm 2.16, \text{ L/h respectively}) \) \( (p < 0.05) \) and medium and low caffeine consumer groups \( (n = 9, 6.77 \pm 3.07 \text{ vs } n = 16, 3.67 \pm 2.08, \text{ L/h respectively}, p < 0.05) \). There was no significant difference between high, medium or low caffeine consumers in the other clearance parameters \( (\text{CL}_{\text{CAPX}} \text{ or } \text{CL}_{\text{PX}}) \) 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 period and no MA period. 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 high caffeine consumers or low caffeine consumers in either period ($p > 0.05$). Among high caffeine consumers, the paraxanthine/caffeine AUC$_{0-24}$ metabolic ratio decreased between the two periods compared to the CL$_{PX}$/CL ratio which increased (Table 3 and Table 4) ($p > 0.05$). The correlation between the paraxanthine/caffeine AUC$_{0-24}$ metabolic ratio and the CL$_{CAPX}$ in the MA period and no MA period was 0.89 and 0.98, respectively.
Discussion

This research has developed a population pharmacokinetic model which 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 modelling indicated there was a significantly lower total apparent caffeine clearance in the no methylxanthine abstinence phase when compared to a 24 h methylxanthine abstinence phase. When stratified by high, medium or low caffeine consumers, >200 mg/day, 100 – 200 mg/day or <100 mg/day, respectively, this difference was only present among high caffeine consumers. 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) while 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 HCC, MCC and LCC, the clearance of caffeine to paraxanthine (CL\text{CAPX}) and the clearance of paraxanthine (CL\text{PX}) did not change between the 24 h methylxanthine abstinence period and no methylxanthine abstinence period, however the total apparent caffeine clearance (CL) which is a sum of all pathways of caffeine elimination was significantly lower in the no MA period among HCC only. This indicates that the activity of CYP1A2 does not differ between the two periods.
and 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, non-linear 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) (Bulitta et al., 2010; Jusko et al., 1976). Numerous studies have been conducted in order to establish the linear concentration range of caffeine in humans, however, conflicting results have been reported (Bonati et al., 1982; Cheng et al., 1990; Denaro et al., 1990; Doude van Troostwijk et al., 2003; Hetzler et al., 1990; Lee et al., 1997; Newton et al., 1981; Nickell & Uhde, 1994). Bonati et al. (1982), described caffeine pharmacokinetics to be linear up to a dose of 10 mg/kg while Newton et al. (1981), also suggested linearity between single doses of 50 and 750 mg in humans (Bonati et al., 1982; Newton et al., 1981). In the present study, the highest consumption of caffeine among all participants was 340 mg/day and based on the previously mentioned studies, 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 & 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 while with low urine flow rate, 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 (Birkett & Miners, 1991; Trang et al., 1985). 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 & Griffin, 2003). However, many of the pharmacodynamic effects, including
blood pressure and norepinephrine concentrations, show acute sensitivity to a caffeine dose following caffeine abstinence (Denaro et al., 1991). Studies have shown that acute ingestion of caffeine (250 – 300 mg), following an abstinence of caffeine for a period of days or weeks results in a short-term stimulation of urine output (Nawrot et al., 2003). It is hypothesised that in our study there is increased sensitivity to the diuretic effect of caffeine, resulting in an increased apparent caffeine clearance, in high caffeine consumers following a 24 h methylxanthine abstinence period. In the no methylxanthine abstinence period, this sensitivity is not present due to the tolerance associated with regular caffeine consumption and therefore the overall apparent caffeine clearance is lower.

The other option that must be considered is the potential changes to formation and elimination of other metabolites which may change the overall caffeine clearance. A study by Kaplan et al., reported a dose-dependent effect of caffeine with a significantly lower clearance of caffeine observed following a 500 mg oral dose of caffeine compared to a 250 mg dose of caffeine (Kaplan et al., 1997). The study found that there was no difference between the paraxanthine AUC0-8h or theophylline AUC0-8h, however a difference was observed for the AUC0-8h of theobromine between the two doses indicating a non-linear pathway associated with formation of theobromine following caffeine consumption. This result is indicative of a change in a pathway other than CYP1A2. Berthou et al. reported that there was a significant interaction between two probe drugs, caffeine and chlorzoxane which were utilised 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 hypothesised that this was due to competitive inhibition, as demonstrated by in vitro data. Similarly, Tang-Liu et al., reported that at least one and possibly
more routes of elimination of caffeine may be saturable and/or its metabolites may inhibit xanthine oxidase (Tang-Liu et al., 1983). 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 modelling results demonstrated that the correlation between the CL<sub>CAPX</sub> and the paraxanthine/caffeine AUC<sub>0-24</sub> metabolic ratio was greater in the no methylxanthine abstinence compared to the 24 h methylxanthine abstinence period. This indicates that paraxanthine/caffeine ratios obtained in the no methylxanthine abstinence period provide better estimates of CYP1A2 activity and variability associated with paraxanthine/caffeine ratios is likely due to the forced 24 h abstinence from caffeine.

There are limitations of this study primarily associated with the model utilised. The final pharmacokinetic model used in this study is non-identifiable in one respect as 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 utilised in this study was considered the best option given the available data in order to generate hypotheses for the changes in pharmacokinetics of caffeine that was observed.

Furthermore, there would be limitations surrounding the parameters of an identifiable model including: 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. The results found in this study could also indicate a change in the bioavailability of caffeine between the two phases of the study. However, previous studies have 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 & Sawers, 1983). There are numerous advantages to this modelling approach. The identification of a single pathway related to the CYP1A2 enzyme pathway (CLCAPX) can be considered a ‘gold standard’ measure of CYP1A2 activity, eliminating the influence of other enzyme pathways which are reflected in the total apparent clearance or metabolic ratios. This model, which was developed based on rich pharmacokinetic data, can be utilised 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 methylxanthine abstinence period has a significant impact on the pharmacokinetics of caffeine in high caffeine consumers. These results have several implications for the utilisation 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 methylxanthine abstinence periods on the pharmacokinetics of caffeine and its metabolites in high caffeine consumers and measure the concentrations of caffeine and metabolites in both plasma and urine.
Acknowledgements

The authors thank Dr Benjamin Guiastrennec for providing the R script to generate the visual predictive checks from the ADAPT code. The authors thank Dr Marilyn Morris and Dr Gauri Rao (Department of Pharmaceutical Sciences, University at Buffalo) for their insight into the results.
Authorship Contribution

**Participated in research design:** Perera, V. Gross, A.S. Xu, H. McLachlan, A.J.

**Conducted experiments:** Perera, V. Xu, H, McLachlan, A.J.

**Contributed new reagents or analytic tools:** McLachlan, A.J.

**Performed data analysis:** Perera, V. Forrest, A. Landersdorfer, C.B.

**Wrote or contributed to the writing of the manuscript:** Perera, V. Forrest, A. Landersdorfer, C.B. Gross, A.S. Ait-Oudhia, S. McLachlan, A.J.
References


Footnotes

Conflict of Interest

The authors declare no conflict of interest
Legend for Figures

**Figure 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 while solid lines represent demethylation pathways. 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

**Figure 2.** Population pharmacokinetic model of caffeine and paraxanthine.

**Figure 3.** Individual model predicted versus observed concentrations (μg/mL) for A) caffeine in the 24 h methylxanthine abstinence phase, B) paraxanthine in the 24 h methylxanthine abstinence phase, C) caffeine in the no methylxanthine abstinence phase and D) paraxanthine in the no methylxanthine abstinence phase

**Figure 4.** Standardized weighted residuals versus time after dose (h) for A) caffeine in the 24 h methylxanthine abstinence phase, B) paraxanthine in the 24 h methylxanthine abstinence phase, C) caffeine in the no methylxanthine abstinence phase and D) paraxanthine in the no methylxanthine abstinence phase

**Figure 5.** Predicted caffeine and paraxanthine concentrations in a representative participant in the A) 24 h methylxanthine abstinence period and B) no methylxanthine abstinence period

**Figure 6.** Visual predictive check for (A) caffeine and (B) paraxanthine in the 24 h methylxanthine abstinence
### Tables

**Table 1. Demographics of participants stratified by caffeine consumption.**

<table>
<thead>
<tr>
<th></th>
<th><strong>High caffeine consumers (n = 5)</strong></th>
<th></th>
<th><strong>Medium caffeine consumers (n = 9)</strong></th>
<th></th>
<th><strong>Low caffeine consumers (n = 16)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>Range (min-max)</td>
<td>Median</td>
<td>Range (min-max)</td>
<td>Median</td>
</tr>
<tr>
<td>Age (years)</td>
<td>23.0</td>
<td>21.0 – 33.0</td>
<td>23.0</td>
<td>19.0 – 32.0</td>
<td>24.0</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>82.9</td>
<td>65.0 – 108.0</td>
<td>79.5</td>
<td>64.9 – 108.0</td>
<td>75.0</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.86</td>
<td>1.67 – 1.92</td>
<td>1.79</td>
<td>1.65 – 1.99</td>
<td>1.76</td>
</tr>
<tr>
<td>Daily Caffeine intake (mg/day)</td>
<td>240.0</td>
<td>205.0 – 340.0</td>
<td>120.0</td>
<td>110 – 155.0</td>
<td>60.0</td>
</tr>
</tbody>
</table>
Table 2. Model parameter estimates in the 24 h methylxanthine abstinence period and no methylxanthine abstinence period

<table>
<thead>
<tr>
<th>Parameter</th>
<th>24 h methylxanthine abstinence period</th>
<th>No methylxanthine abstinence period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Population Mean Estimate</td>
<td>RSE %</td>
</tr>
<tr>
<td>ka (h⁻¹)</td>
<td>3.65</td>
<td>41.6</td>
</tr>
<tr>
<td>k₁₂ (h⁻¹)</td>
<td>1.71</td>
<td>19.4</td>
</tr>
<tr>
<td>k₂₁ (h⁻¹)</td>
<td>2.02</td>
<td>14.4</td>
</tr>
<tr>
<td>V₁ (L)</td>
<td>31.2</td>
<td>38.8</td>
</tr>
<tr>
<td>V₂ (L)</td>
<td>24.4</td>
<td>47.2</td>
</tr>
<tr>
<td>Tlag (h⁻¹)</td>
<td>0.15</td>
<td>54.5</td>
</tr>
<tr>
<td>CLCAPX (L/h)</td>
<td>2.73</td>
<td>15.0</td>
</tr>
<tr>
<td>CLPX (L/h)</td>
<td>3.81</td>
<td>11.2</td>
</tr>
<tr>
<td>CLCAO (L/h)</td>
<td>4.86</td>
<td>40.1</td>
</tr>
<tr>
<td>IC(1) (mg)</td>
<td>1.72</td>
<td>82.3</td>
</tr>
<tr>
<td>IC(2) (mg)</td>
<td>1.20</td>
<td>Fixed</td>
</tr>
<tr>
<td>IC(3) (mg)</td>
<td>4.06</td>
<td>22.8</td>
</tr>
<tr>
<td>IC(4) (mg)</td>
<td>1.37</td>
<td>Fixed</td>
</tr>
<tr>
<td>SD1int</td>
<td>0.05</td>
<td>Fixed</td>
</tr>
<tr>
<td>SD1slope</td>
<td>0.02</td>
<td>38.8</td>
</tr>
<tr>
<td>SD2int</td>
<td>0.05</td>
<td>Fixed</td>
</tr>
<tr>
<td>SD2slope</td>
<td>0.04</td>
<td>32.2</td>
</tr>
</tbody>
</table>

RSE% - Relative standard error of parameter as a percentage [represents the precision of the parameter estimate]; CV% - coefficient of variation percent [represents the true biological variability between the participants]; IC(i) – Initial condition in i th compartment; SD1int – variance model parameter describing additive residual error of caffeine; SD1slope – variance model parameter describing the proportional residual error of caffeine; SD2int – variance model parameter describing additive residual error of paraxanthine; SD2slope – variance model parameter describing proportional residual error of paraxanthine.
Table 3. Population clearance parameters of caffeine and paraxanthine in low, medium and high caffeine consumers.

<table>
<thead>
<tr>
<th></th>
<th>Low Caffeine consumers (n = 16) (0 – 100mg/day)</th>
<th>Medium Caffeine consumers (n = 9) (100 – 200 mg/day)</th>
<th>High Caffeine consumers (n = 5) (&gt;200 mg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h Methylxanthine abstinence</td>
<td>No Methylxanthine abstinence</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Average Mean ± SD 95% CI</td>
<td>Average Mean ± SD 95% CI</td>
<td></td>
</tr>
<tr>
<td>Total clearance of caffeine (CL, L/h)</td>
<td>6.88 ± 2.40 5.61 – 8.16</td>
<td>6.22 ± 2.33 4.97 – 7.46</td>
<td>3.40 – 9.20 &lt;0.05</td>
</tr>
<tr>
<td>Clearance of caffeine to paraxanthine (CL\textsubscript{CAPX} L/h)</td>
<td>2.61 ± 0.55 2.31 – 2.90</td>
<td>2.55 ± 0.45 2.31 – 2.79</td>
<td>2.31 – 3.10 NS</td>
</tr>
<tr>
<td>Clearance of caffeine to other metabolites plus renal clearance (CL\textsubscript{CAO} L/h)</td>
<td>4.28 ± 1.93 3.25 – 5.31</td>
<td>3.67 ± 2.08 2.56 – 4.78</td>
<td>3.66 – 4.19 NS</td>
</tr>
<tr>
<td>Clearance of paraxanthine (CL\textsubscript{PX} L/h)</td>
<td>3.77 ± 0.30 3.61 – 3.93</td>
<td>3.70 ± 0.18 3.60 – 3.80</td>
<td>3.78 ± 0.26 NS</td>
</tr>
<tr>
<td>Fraction metabolized by CYP1A2 pathway (CL\textsubscript{PX}/ CL)</td>
<td>0.61 ± 0.21 0.50 – 0.72</td>
<td>0.68 ± 0.26 0.54 – 0.81</td>
<td>0.42 ± 0.19 0.19 – 0.65 &lt;0.05</td>
</tr>
</tbody>
</table>

The p-values reflect paired samples t-test between the two periods of the same metrics. All clearances are expressed in L/h.
Table 4. Area under the curve (AUC) values from population model stratified by high, medium or low caffeine consumption and abstinence period.

<table>
<thead>
<tr>
<th></th>
<th>Low Caffeine consumers (n = 16)</th>
<th>Medium Caffeine consumers (n = 9)</th>
<th>High Caffeine consumers (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h Methylxanthine abstinence</td>
<td>No Methylxanthine abstinence</td>
<td>Significance*</td>
</tr>
<tr>
<td></td>
<td>Mean ± SD  95% CI</td>
<td>Mean ± SD  95% CI</td>
<td></td>
</tr>
<tr>
<td>AUC$_{0-24}$ of Caffeine</td>
<td>15.17 ± 4.45  12.80 – 17.55</td>
<td>17.11 ± 6.78  13.50 – 20.72</td>
<td>NS</td>
</tr>
<tr>
<td>AUC$_{0-24}$ of Paraxanthine</td>
<td>9.92 ± 2.35  8.67 – 11.17</td>
<td>10.15 ± 3.68  8.19 – 12.11</td>
<td>NS</td>
</tr>
<tr>
<td>AUC$<em>{0-24}$/AUC$</em>{0-24}$ of Caffeine (AUC Metabolic ratio)</td>
<td>0.60 ± 0.15  0.60 – 0.76</td>
<td>0.61 ± 0.12  0.54 – 0.68</td>
<td>NS</td>
</tr>
<tr>
<td>AUC$_{0-24}$ of Caffeine</td>
<td>10.96 ± 4.03  7.86 – 14.06</td>
<td>10.74 ± 3.26  8.24 – 13.25</td>
<td>NS</td>
</tr>
<tr>
<td>AUC$_{0-24}$ of Paraxanthine</td>
<td>7.60 ± 2.41  5.74 – 9.55</td>
<td>7.20 ± 1.34  6.18 – 8.24</td>
<td>NS</td>
</tr>
<tr>
<td>AUC$<em>{0-24}$/AUC$</em>{0-24}$ of Caffeine (AUC Metabolic ratio)</td>
<td>0.71 ± 0.12  0.62 – 0.80</td>
<td>0.70 ± 0.13  0.60 – 0.79</td>
<td>NS</td>
</tr>
<tr>
<td>AUC$_{0-24}$ of Caffeine</td>
<td>10.75 ± 4.45  5.22 – 16.27</td>
<td>16.87 ± 6.37  8.96 – 24.78</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>AUC$_{0-24}$ of Paraxanthine</td>
<td>7.52 ± 1.64  5.49 – 9.56</td>
<td>10.24 ± 2.59  7.02 – 13.45</td>
<td>NS</td>
</tr>
<tr>
<td>AUC$<em>{0-24}$/AUC$</em>{0-24}$ of Caffeine (AUC Metabolic ratio)</td>
<td>0.75 ± 0.16  0.55 – 0.94</td>
<td>0.63 ± 0.10  0.50 – 0.76</td>
<td>NS</td>
</tr>
</tbody>
</table>

The p-values reflect paired samples t-test between the two periods of the same metrics. The units of AUC are ug/mL.h.
Figure 2

Bolus Oral Dose
(100 mg of caffeine)

\[ \text{ka} - \text{rate constant from absorptive compartment; } k_{12} - \text{rate constant from compartment 1 of caffeine to peripheral compartment; } k_{21} - \text{rate constant from peripheral compartment to caffeine central compartment; } V_1 - \text{volume of distribution of caffeine in compartment 1; } V_2 - \text{volume of distribution of paraxanthine in compartment 2; } T_{lag} - \text{lag time from absorptive compartment; } CL_{CAPX} - \text{Clearance of caffeine to paraxanthine; } CL_{PK} - \text{Clearance of paraxanthine; } CL_{C4O} - \text{renal clearance of caffeine plus metabolic clearance of caffeine to theophylline and theobromine; } F - \text{bioavailability of caffeine (fixed at 1); } Fm - \text{Fraction of parent drug converted to metabolite (not estimated). } X_2 - \text{peripheral compartment; } X_4 - \text{absorptive compartment.} \]

All distribution and elimination parameters of caffeine are predicated on F while all distribution and elimination parameters of paraxanthine are predicated on Fm.
Figure 5

A. Caffeine – 24 h methylxanthine abstinence

B. Paraxanthine – 24 h methylxanthine abstinence

C. Caffeine – No methylxanthine abstinence

D. Paraxanthine – No methylxanthine abstinence