

## PHARMACOKINETICS

# Population pharmacokinetics of caffeine in healthy male adults using mixed-effects models

K.-Y. Seng\* PhD, C.-Y. Fun† BSc, Y.-L. Law† MSc, W.-M. Lim‡ MBBS, W. Fan† BSc and C.-L. Lim† PhD

\*Bioengineering Laboratory, Defence Medical and Environmental Research Institute, DSO National Laboratories, †Military Physiology Laboratory, Defence Medical and Environmental Research Institute, DSO National Laboratories, and ‡Performance Maximization Branch, HQ Air Force Medical Service, Republic of Singapore Air Force, Singapore

## SUMMARY

**Objective:** Caffeine has been shown to maintain or improve the performance of individuals, but its pharmacokinetic profile for Asians has not been well characterized. In this study, a population pharmacokinetic model for describing the pharmacokinetics of caffeine in Singapore males was developed. The data were also analysed using non-compartmental models.

**Methods:** Data gathered from 59 male volunteers, who each ingested a single caffeine capsule in two clinical trials (3 or 5 mg/kg), were analysed via non-linear mixed-effects modelling. The participants' covariates, including age, body weight, and regularity of caffeinated-beverage consumption or smoking, were analysed in a stepwise fashion to identify their potential influence on caffeine pharmacokinetics. The final pharmacostatistical model was then subjected to stochastic simulation to predict the plasma concentrations of caffeine after oral (204, 340 and 476 mg) dosing regimens (repeated dosing every 6, 8 or 12 h) over a hypothetical 3-day period.

**Results:** The data were best described by a one-compartmental model with first-order absorption and first-order elimination. Smoking status was an influential covariate for clearance:  $\text{clearance (mL/min)} = 110 \times \text{SMOKE} + 114$ , where SMOKE was 0 and 1 for the non-smoker and the smoker respectively. Interoccasion variability was smaller

compared to interindividual variability in clearance, volume and absorption rate (27% vs. 33%, 10% vs. 15% and 23% vs. 51% respectively). The extrapolated elimination half-lives of caffeine in the non-smokers and the smokers were  $4.3 \pm 1.5$  and  $3.0 \pm 0.7$  h respectively. Dosing simulations indicated that dosing regimens of 340 mg (repeated every 8 h) and 476 mg (repeated every 6 h) should achieve population-averaged caffeine concentrations within the reported beneficial range ( $4.5\text{--}9 \mu\text{g/mL}$ ) in the non-smokers and the smokers respectively over 72 h.

**Conclusion:** The population pharmacokinetic model satisfactorily described the disposition and variability of caffeine in the data. Mixed-effects modelling showed that the dose of caffeine depended on cigarette smoking status.

**Keywords:** caffeine, NONMEM, pharmacokinetics, population modelling, stochastic simulation

## INTRODUCTION

Ergogenic aids such as caffeine may alleviate sleepiness following bouts of sleep deprivation or loss (1, 2). Caffeine suppresses the drive for sleep by stimulating neurons involved in arousal and inhibiting neurons involved in sleep (3, 4). In this regard, caffeine has been shown to maintain or improve the performance quality of sleep-restricted individuals, e.g. military personnel or vehicle operators during prolonged or sustained operations (5, 6).

As with most drugs, the beneficial effects of caffeine are present across a limited range of plasma concentrations. Given that caffeine is frequently consumed in the form of food, beverages

Received 24 June 2008, Accepted 4 August 2008

Correspondence: K.-Y. Seng, Bioengineering Laboratory, Defence Medical and Environmental Research Institute, DSO National Laboratories, 27 Medical Drive, #09-01, Singapore 117510, Singapore. Tel.: +65 6485 7107; fax: +65 6485 7172; e-mail: skokyong@dso.org.sg

or cigarettes, habitual caffeine use may have a large impact on the resultant plasma and tissue levels in individuals, which could translate into varied caffeine response. Although the effect of smoking on caffeine disposition and elimination has been studied (7, 8), albeit using non-compartmental modelling methods, there are limited pharmacokinetic studies on the concomitant influences of caffeine consumption via caffeinated beverages and cigarettes on plasma caffeine pharmacokinetics in humans. These sparse data make dosing problematic. Extrapolation of dosing guidelines from data for non-smoker, non-caffeine takers may put regular smokers and/or caffeine takers at risk for inadequate effect or increased toxicity.

The objective of this study was to develop a population pharmacokinetic model for describing the pharmacokinetics of caffeine in healthy young adults who display different levels of caffeine habituation as a result of drinking caffeine-containing beverages or cigarette smoking. The results bear directly on the question of which of these two means of caffeine consumption, if any, alters caffeine disposition and elimination more significantly. In addition, information stemming from the population pharmacokinetic data analysis would permit individual-specific factors, which contribute to the variability in caffeine pharmacokinetics, and covariates, which could be used for dose calculation considerations, to be identified. Furthermore, interoccasion variability (IOV), i.e. intraindividual variations of the pharmacokinetic parameters during different days of dosage administration, needed quantification. The final model was then subjected to stochastic simulations to predict caffeine pharmacokinetics under different dosage amounts and frequencies.

## MATERIALS AND METHODS

### *Study design*

The clinical study was designed as a prospective, single-centre, non-randomized, open-label investigation of the pharmacokinetics of caffeine in male volunteers. The subjects were judged to be healthy by a complete medical history, physical examination, and normal haematological and biochemical values. For pharmacokinetic profiling (see below), volunteers fasted for 6 h prior to dosing and were

permitted to begin eating 2 h afterward. During the study, an identical diet was provided to all participants. All participants were orally dosed with, first, 3 mg/kg body weight of caffeine and then 5 mg/kg body weight of caffeine. The two separate clinical trials – which constituted two different occasions – for each participant was separated by a washout period of no less than 7 days. Consumption of alcohol and caffeine-containing products was prohibited 96 h before and during each clinical trial. The study was approved by an institutional review board, and written informed consent was obtained from all participants.

### *Volunteers*

Fifty-nine healthy adult Asian volunteers were included. In what follows, a non-caffeine consumer refers to an individual who consumed beverages (coffee or tea) containing  $\leq 200$  mg of caffeine per day, and a regular caffeine consumer to one who consumed more  $> 200$  mg. A cup (150 mL) of coffee or tea is equivalent to approximately a caffeine dose of 70 and 25 mg respectively (9). Additionally, a non-smoker refers to an individual who did not smoke for at least 6 months prior to dosing. The study population consisted of 14 non-caffeine consumers, non-smokers (NCNS) [age (mean  $\pm$  SD)  $21 \pm 2$  years, body weight  $62 \pm 9$  kg], 15 caffeine consumers, non-smokers (CNS) (age  $24 \pm 4$  years, body weight  $69 \pm 12$  kg), and 30 caffeine consumer, smokers (CS) (actual consumption  $10 \pm 4$  cigarettes daily, age  $23 \pm 5$  years, body weight  $71 \pm 19$  kg).

### *Administration of study drug*

Caffeine was given orally in the form of anhydrous caffeine capsules (Union Chemical and Pharmaceutical Pte Ltd, Singapore); the capsules were available in 5, 10, 20, 50, 200, 250, 300 and 400 mg. The actual dosages that were administered to the participants for 3 and 5 mg/kg regimens were  $205 \pm 47$  and  $342 \pm 79$  mg respectively. Dosage administration was observed and recorded for each participant.

### *Pharmacokinetic sampling*

For each clinical trial, blood samples (5 mL each) were withdrawn just prior to administration of the

oral dose of caffeine and at 10 min, 20 min, 30 min, 60 min, 2 h, 4 h, 6 h, 12 h and 24 h post-dose. Ammonium sulphate (600 mg) was added to each collected sample immediately after its collection from the subjects. After 1 min vigorous shaking (Vortex-2 Genie Shaker; Scientific Industries Inc., Bohemia, NY, USA), a liquid-liquid extraction was applied to the sample using 6 mL of a solution containing ethyl acetate and isopropyl alcohol (8/1; v/v). After vigorous shaking for 2 min, the sample was centrifuged at 6000 g for 10 min. The organic phase was separated and stored in liquid nitrogen ( $-85^{\circ}\text{C}$ ). The plasma samples were packed into boxes kept at  $0-4^{\circ}\text{C}$  and transported to a dedicated laboratory for caffeine concentration assay within two weeks after blood were sampled.

### Caffeine measurements via HPLC

Caffeine concentration in  $5\ \mu\text{L}$  of the plasma samples was measured by high-performance liquid chromatography (HPLC). The separation was performed on a Waters Atlantis dC<sub>18</sub> column ( $150 \times 1\ \text{mm}$  i.d.,  $5\text{-}\mu\text{m}$  particle size) (Milford, Wilford, MA, USA) with the use of an isocratic mobile phase consisting of methanol/ $0.1\ \text{M}$   $\text{NaH}_2\text{PO}_4$  buffer (pH 7.4). The flow rate and column temperature were maintained at  $70\ \mu\text{L}/\text{min}$  and  $40^{\circ}\text{C}$  respectively. The limit of detection was  $0.1\ \mu\text{g}/\text{mL}$  using a UV detector at  $274\ \text{nm}$ . Precision and accuracy were evaluated by replicate analysis of spiked plasma samples at three concentrations that spanned the concentration range ( $0.5\text{--}30\ \mu\text{g}/\text{mL}$ ) in clinical study samples. Within-run precision (coefficient of variation,  $n = 6$ ) ranged from  $0.44\%$  to  $5.85\%$ . Accuracy (percentage of expected values;  $n = 6$ ) ranged from  $95$  to  $103\%$ . All concentration measurements were analysed using the same analytical protocol.

### Population pharmacokinetic analysis

The population pharmacokinetics of caffeine were determined by using NONMEM (version VI, ADVAN2, TRANS2; NONMEM Project Group, University of California, San Francisco, CA, USA), interfaced with PDx-Pop version 2.2a (GloboMax LLC, Hanover, MD, USA) in conjunction with a G77 compiler. A one-compartment model with first-order absorption and first-order elimination was fitted to the data, using first-order conditional

estimation with interaction. For parameter estimation, the model was parameterized for absorption rate ( $K_a$ ), clearance ( $CL/F$ ) and volume of distribution ( $V/F$ ). Following Beal (10), measurements that were below the limit of detection were replaced with a value equal to half the detection limit prior to the NONMEM analysis.

### Between-subject and between-occasion variance models

The interindividual variability (IIV) was modelled, assuming a log-normal distribution, as follows:

$$\begin{aligned} CL/F_{ij} &= CL/F \cdot e^{(\eta_{i,CL/F} + K_{j,CL/F})} \\ V/F_{ij} &= V/F \cdot e^{(\eta_{i,V/F} + K_{j,V/F})} \\ K_{a,ij} &= K_a \cdot e^{(\eta_{i,K_a} + K_{j,K_a})} \end{aligned} \quad (1)$$

where  $CL/F_{ij}$ ,  $V/F_{ij}$  and  $K_{a,ij}$  denoted the parameters for the  $i$ th subject on the  $j$ th occasion about the typical respective population values  $CL/F$ ,  $V/F$  and  $K_a$  respectively. The parameters  $\eta_{i,CL/F}$ ,  $\eta_{i,V/F}$  and  $\eta_{i,K_a}$  were random variables distributed with means of 0 and respective variances of  $\omega_{CL/F}^2$ ,  $\omega_{V/F}^2$  and  $\omega_{K_a}^2$ .  $\kappa$  (kappa) was a random variable representing the variability of a given pharmacokinetic parameter value on different occasions (in this study, there were two occasions with administered dosages of 3 and  $5\ \text{mg}/\text{kg}$ ). The interoccasion variability (IOV) was assumed to be sampled from a normal distribution having a mean of 0 and a variance of  $\pi^2$ . In modelling the IOV, it was assumed that the variances of each parameter were sampled from the same distribution.

### Residual unknown variance model

Various residual unknown variance models were tested: an additive error model, a proportional error model and a combined proportional-additive error model:

$$C_{ij}^{\text{obs}} = C_{ij}^{\text{pred}} + \varepsilon_{ij}; \text{ Additive } (2a)$$

$$C_{ij}^{\text{obs}} = C_{ij}^{\text{pred}} (1 + \varepsilon_{ij}); \text{ Proportional } (2b)$$

$$C_{ij}^{\text{obs}} = C_{ij}^{\text{pred}} (1 + \varepsilon_{ij}^P) + \varepsilon_{ij}^A; \text{ Proportional-additive } (2c)$$

where  $C_{ij}^{\text{pred}}$  was the  $j$ th blood concentration of the  $i$ th individual predicted by the pharmacokinetic

model,  $C_{ij}^{\text{obs}}$  was measured concentration and  $\epsilon_{ij}$  represented the residual departure of the model from the  $j$ th observation available from the  $i$ th individual.  $\epsilon_{ij}$  was a normally distributed random variable with zero mean and variance  $\sigma^2$ . The superscripts  $P$  and  $A$  on  $\epsilon_{ij}$  values in (2c) denoted proportional and additive respectively.

### *Covariate model*

An initial analysis was conducted by permitting NONMEM to estimate the base model parameters (i.e. no covariates). Once the final variance model was selected, covariate models were tested to assess the potential influence of covariates (i.e. age, body weight, consumption of caffeine beverages and cigarette smoking) on caffeine disposition. Individual parameter estimates of  $K_a$ ,  $CL/F$  and  $V/F$  were obtained using the POSTHOC option in NONMEM. Scatter plots of these pharmacokinetic parameters against each covariate were examined for trends. Covariates, identified visually as potential factors influencing the pharmacokinetics of caffeine, were then tested formally using the stepwise approach. The influence of the mean-centred continuous variables and the categorical variables was assessed by adding these to the base model in turn and noting the change in the objective function value (OFV). The inclusion of a covariate improved the fit of the data to the model if there was a decrease in the OFV. The difference between a pair of OFV values when a covariate was included (full model) and then excluded (reduced model) was tested for significance ( $\alpha = 0.05$ ), using the chi-square statistic with 1 degree of freedom ( $\chi^2_{1,0.05} = 3.84$ ).

### *Model assessment*

The final model was assessed by an inspection of standard diagnostic plots of observed concentration versus population model predicted concentration and separate plots of weighted residual versus model-predicted concentration, elapsed time, subject identification, and screened covariates (11). A degenerate visual predictive check was performed by simulating, using the SIML function in NONMEM, from the final model 500 concentrations at predetermined sampling

times during 0–24 h. The 50th percentile concentration (as an estimator of the population-predicted concentration) and the 5th and 95th percentile concentrations were processed and then plotted against elapsed time. Observed caffeine concentrations were compared with the predictions.

### *Concentration simulation*

Using the reported beneficial window of 4–9  $\mu\text{g/mL}$  for caffeine (12, 13) as a guide, stochastic simulations were used to separately predict the concentrations of caffeine after multiple oral doses of 204, 340 or 476 mg (repeated dosing every 6, 8 or 12 h) over a time period of 72 h. This was performed to determine suitable dosing regimens that could be utilized in a clinical setting; the 72-h time period was arbitrarily chosen to typify a sustained period of activity. The simulations were performed by applying doses derived from multiplying 3, 5 and 7 mg/kg, respectively, by the median weight (68 kg) of the tested individuals in the present study. The plasma caffeine concentrations were simulated in 500 healthy young Asian adult males based on the values of the fixed-effects parameters and the variances in the final model for caffeine. For all simulations, the median, and 5th and 95th percentiles of caffeine concentrations were analysed.

### *Non-compartmental pharmacokinetic analysis*

The pharmacokinetic parameters of caffeine in each of the study participants were analysed by non-compartmental analysis using WINNONLIN (version 2.1; Pharsight Corporation, Palo Alto, CA, USA). The area under the plasma concentration-time curves ( $AUC_{0-t}$ ), defined as the area under the concentration-time curve from the time of dose until the last measurable concentration, was calculated by the trapezoidal method. The terminal half-life ( $t_{1/2}$ ) was calculated as  $0.693/k$ , and  $k$  was the slope of the terminal regression line. The estimated pharmacokinetic parameters for the NCNS, CNS and CS groups under 3 and 5 mg/kg dosage regimens were compared using the Mann–Whitney two sample rank-sum test in the open-source statistical software R.



## RESULTS

### Non-compartmental pharmacokinetic analysis

Caffeine concentration measurements from all but three participants were used for individual non-compartmental pharmacokinetic analysis. Due to insufficient data ( $\leq 3$  plasma concentration-time points), non-compartmental pharmacokinetic analysis resulted in model non-convergence for these three participants (one CNS volunteer and two CS volunteers under the 3 mg/kg dosage regimen). In Fig. 1, we show the mean ( $\pm$ SD) plasma concentration-time profiles under the 3 and 5 mg/kg dosage regimens. The estimated mean ( $\pm$ SD)  $CL$  (clearance),  $V_{ss}$  (steady-state volume of distribution),  $t_{1/2}$  and  $AUC_{0-t}$  are summarized in Table 1.

As shown in Table 1, for both the dosage regimens,  $CL$  was statistically significantly larger in the CS group compared to those in the NCNS and CNS groups ( $P < 0.05$ ). This difference reflected mainly a decrease in  $AUC_{0-t}$  in the CS group [ $23.1 \pm 16.72$  (CS) vs.  $46.58 \pm 13.65$  (NCNS) and  $50.9 \pm 34.05$  h  $\mu\text{g/mL}$  (CNS) for 3 mg/kg;  $46.06 \pm 18.92$  (CS) vs.  $65.85 \pm 44$  (NCNS) and  $72.59 \pm 44.08$  h  $\mu\text{g/mL}$  (CNS) for 5 mg/kg;  $P < 0.05$ ]. No statistically significant difference in  $V_{ss}$  between NCNS, CNS and CS subjects within each dosage regimen was observed. By and large, our results indicated that higher caffeine doses led to statistically significantly longer  $t_{1/2}$  [e.g.  $5.21 \pm 1.08$  (5 mg/kg) vs.  $4.21 \pm 1.42$  (3 mg/kg) for CS group;  $P < 0.05$ ] and lower  $CL$  [e.g.  $1.01 \pm 0.77$  (5 mg/kg) vs.  $1.81 \pm 0.67$  (3 mg/kg) for NCNS group;  $P < 0.05$ ]. As reported by (14), this is largely because caffeine metabolism in humans exhibits saturable kinetics, starting from as low a plasma concentration as  $45 \mu\text{M}$  ( $8.74 \mu\text{g/mL}$ ).

### Population pharmacokinetic analysis

A total of 870 caffeine concentration measurements were taken and made available for analysis in this study. Summary results of the population model-building process are shown in Table 2. The data did not support the inclusion of an absorption lag time in any model. Neither age nor body weight on  $CL/F$ ,  $V/F$  and  $K_a$  significantly improved the fit, nor did consumption of caffeine beverages as indicator variable. The

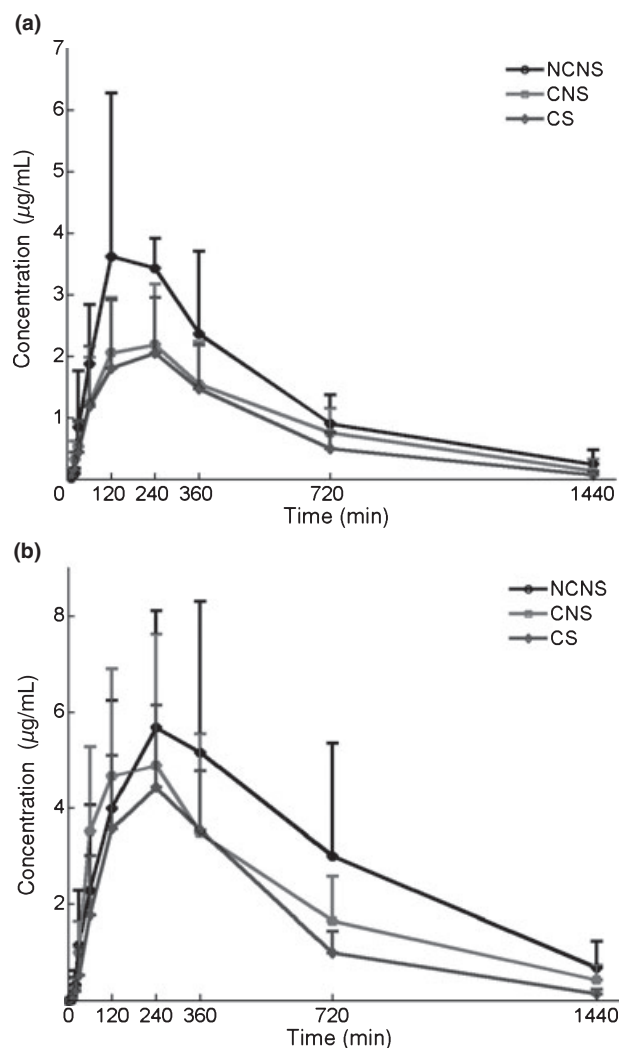


Fig. 1. Plasma concentration-time curves (mean ( $\pm$ SD)) for caffeine for volunteers receiving 3 (a) and 5 (b) mg/kg oral doses of caffeine. The results are shown for the NCNS, CNS and CS groups.

linear positive influence of the categorical variable cigarette smoking on  $CL/F$  is shown in Fig. 2. Inclusion of cigarette smoking as a covariate on  $CL/F$  significantly decreased the OFV by 97. These findings indicated that smoking status was the single most important factor to influence caffeine pharmacokinetics in the present study. Furthermore, inclusion of the IOV for  $CL/F$ ,  $V/F$  and  $K_a$  reduced the OFV further, to 132. The RUV was best modelled by using a combined proportional and additive model, as seen by an increase in the OFV and by numerical difficulties when the additive and proportional models were used separately.

**Table 1.** Noncompartmental pharmacokinetic parameters of caffeine in NCNS, CNS and CS groups after a single oral dose of 3 and 5 mg/kg was administered (mean  $\pm$  sd)

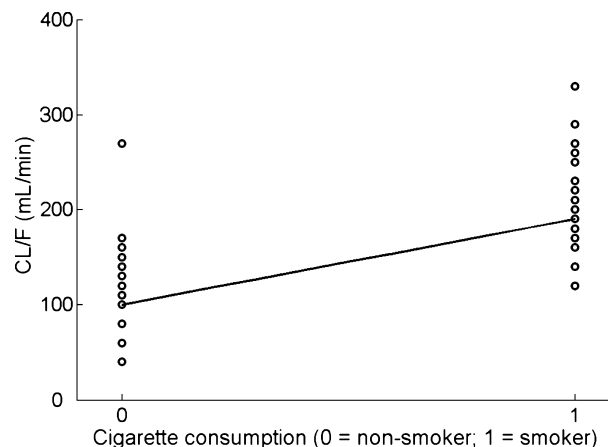
	CL			$V_{ss}$		$AUC_{0-t}$ (h $\mu$ g/mL)
	mL/min	mL/min/kg	$t_{1/2}$ (h)	L	L/kg	
3 mg/kg						
NCNS	80.74 $\pm$ 43.06	1.81 $\pm$ 0.67 <sup>d</sup>	4.79 $\pm$ 2.25	51.25 $\pm$ 28.44	0.85 $\pm$ 0.48	46.58 $\pm$ 13.65
CNS	82.7 $\pm$ 43.25	1.21 $\pm$ 0.56 <sup>c,d</sup>	4.86 $\pm$ 1.59 <sup>d</sup>	40.21 $\pm$ 30.05	0.95 $\pm$ 0.46 <sup>d</sup>	50.9 $\pm$ 34.05
CS	187.55 $\pm$ 104.48 <sup>a,b,d</sup>	2.81 $\pm$ 1.76 <sup>a,b,d</sup>	4.21 $\pm$ 1.42 <sup>d</sup>	49.33 $\pm$ 51.19	1.05 $\pm$ 0.85 <sup>d</sup>	23.1 $\pm$ 16.72 <sup>a,b,d</sup>
5 mg/kg						
NCNS	82.29 $\pm$ 56.51	1.01 $\pm$ 0.77 <sup>d</sup>	5.12 $\pm$ 3.03	44.18 $\pm$ 32.58	0.72 $\pm$ 0.56	65.85 $\pm$ 44
CNS	81.85 $\pm$ 52.37	1.11 $\pm$ 0.89 <sup>d</sup>	5.56 $\pm$ 2.16 <sup>d</sup>	47.42 $\pm$ 48.21	0.66 $\pm$ 0.7 <sup>d</sup>	72.59 $\pm$ 44.08
CS	132.97 $\pm$ 51.01 <sup>a,b,d</sup>	2.01 $\pm$ 0.9 <sup>a,b,d</sup>	5.12 $\pm$ 1.08 <sup>d</sup>	48.84 $\pm$ 25.16	0.75 $\pm$ 0.43 <sup>d</sup>	46.06 $\pm$ 18.92 <sup>a,b,d</sup>

<sup>a</sup>Statistical significance between pharmacokinetic parameters between CS group and NCNS group within each dosage regimen.<sup>b</sup>Statistical significance between pharmacokinetic parameters between CS group and CNS group within each dosage regimen.<sup>c</sup>Statistical significance between pharmacokinetic parameters between NCNS group and CNS group within each dosage regimen.<sup>d</sup>Statistical significance between values of the same pharmacokinetic parameter between 3 and 5 mg/kg dosage regimens.**Table 2.** Development of structural model for pharmacokinetics of caffeine

Model	Parameterization <sup>a</sup>	$\Delta$ OFV <sup>b</sup>
1	$CL/F = \theta_1$ ; $V/F = \theta_2$ ; $K_a = \theta_3$	
2	$CL/F = \theta_1 * WT + \theta_2$ ; $V/F = \theta_3 * WT + \theta_4$ ; $K_a = \theta_5$	-5 <sup>c</sup>
3	$CL/F = \theta_1 * (WT/68) + \theta_2$ ; $V/F = \theta_3 * (WT/68) + \theta_4$ ; $K_a = \theta_5$	+3
4	$CL/F = \theta_1 * AGE + \theta_2$ ; $V/F = \theta_3 * AGE + \theta_4$ ; $K_a = \theta_5$	0
5	$CL/F = \theta_1 * CAFF + \theta_2$ ; $V/F = \theta_3 * CAFF + \theta_4$ ; $K_a = \theta_5$	-1 <sup>c</sup>
6	$CL/F = \theta_1 * SMOK + \theta_2$ ; $V/F = \theta_3 * SMOK + \theta_4$ ; $K_a = \theta_5$	-83
7	$CL/F = \theta_1$ ; $V/F = \theta_2 * SMOK + \theta_3$ ; $K_a = \theta_4$	-78
8 <sup>d</sup>	$CL/F = \theta_1 * SMOK + \theta_2$ ; $V/F = \theta_3$ ; $K_a = \theta_4$	-97

<sup>a</sup>WT, body weight (kg); WT/68, body weight (kg) centred on median weight (68 kg); AGE, age (years); CAFF, caffeine beverage consumption (0 = less than or equal to 200 mg of caffeine in caffeine-based beverages per day, 1 = more than 200 mg of caffeine in caffeine-based beverages per day); SMOKE, cigarette consumption (0 = non-smoker, 1 = smoker).<sup>b</sup> $\Delta$ OFV, change in OFV from that of model 1 (OFV = 249).<sup>c</sup>Occurrence of rounding errors during fitting.<sup>d</sup>Final model.

Parameter values for the final population model are shown in Table 3. For the non-smokers and smokers in the present study, the estimated mean

**Fig. 2.** Relationship of cigarette consumption to individual estimates of  $CL/F$  for caffeine.

( $\pm$ SD) times ( $T_{max}$ ) for peak concentration to occur after a dose were  $3.2 \pm 1.0$  and  $3.0 \pm 0.61$  h respectively, calculated from each subject's conditional estimates of  $K_a$  and  $K_e$  by the standard formula  $T_{max} = \ln(K_a/K_e)/(K_a - K_e)$  for a one-compartment extravascular model. The  $T_{max}$  values in the non-smokers and the smokers were not significantly different ( $P = 0.381$ ). Typical values for  $CL/F$  calculated from conditional estimates for the non-smokers and the smokers were  $120.87 \pm 45.32$  and  $210.74 \pm 57.2$  mL/min respectively. There were statistically significant differences in the values of  $CL/F$  in the non-smokers and the smokers ( $P < 0.001$ ). The typical values of  $V/F$  and  $K_a$  over

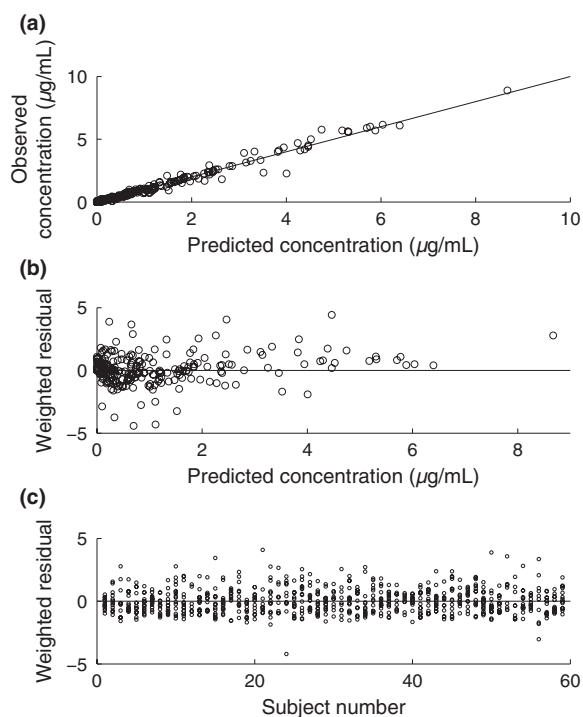
**Table 3.** Final parameter estimates. Values in parentheses are relative standard errors (in per cent) of the estimates

Parameter and model	Final model value
Structural model <sup>a</sup>	
$\theta_1$ (mL/min)	110 (18.3)
$\theta_2$ (mL/min)	114 (8.6)
$\theta_3$ (L)	41.50 (8.8)
$\theta_4$ (L/min)	0.0116 (10.4)
Variance model	
IIV <sub>CL/F</sub> (CV%)	33.2 (35.4)
IIV <sub>V/F</sub> (CV%)	15.2 (15.1)
IIV <sub>Ka</sub> (CV%)	51.3 (51)
IOV <sub>CL/F</sub> (CV%)	26.2 (27)
IOV <sub>V/F</sub> (CV%)	10.3 (54.8)
IOV <sub>Ka</sub> (CV%)	22.9 (23.3)
RUV (CV%)	0.144 (20.3)
RUV ( $\mu\text{g/mL}$ )	0.0103 (28.3)

<sup>a</sup> $CL/F = \theta_1 \cdot \text{SMOK} + \theta_2$ ;  $V/F = \theta_3$ ;  $K_a = \theta_4$ .

all subjects were  $46.31 \pm 6.35$  L and  $0.0107$  per minute respectively. The IIV about  $CL/F$ ,  $V/F$  and  $K_a$  was 33%, 15%, and 51% respectively. The IOV for  $CL/F$ ,  $V/F$  and  $K_a$  were 27%, 10%, and 23% respectively. The  $t_{1/2}$ 's, derived from the expression  $t_{1/2} = (0.693 \cdot V/F)/(CL/F)$  with individual estimates of  $CL/F$  and  $V/F$ , were  $4.31 \pm 1.45$  and  $2.97 \pm 0.68$  h for the non-smokers and the smokers respectively. The  $t_{1/2}$ 's between the two subgroups were statistically significant at  $\alpha = 0.05$ .

The observed vs. population model-predicted concentrations are presented in Fig. 3a. Weighted residuals vs. population model-predicted values (Fig. 3b) showed points that are symmetrically distributed about the null ordinate indicating a good fit of the model to the data. Similarly, points in the plot of weighted residual vs. subject number (Fig. 3c) were distributed symmetrically in a band, which mostly were within 3 units of the null ordinate, indicating that data from any individual did not contribute to marked deviation from the model. The degenerate visual predictive check showed the observed data from the non-smokers and smokers to be symmetrically distributed about their respective 50th percentile profiles, with approximately 15% of the data distributed outside the 5th- to 95th-percentile boundaries (data not shown).



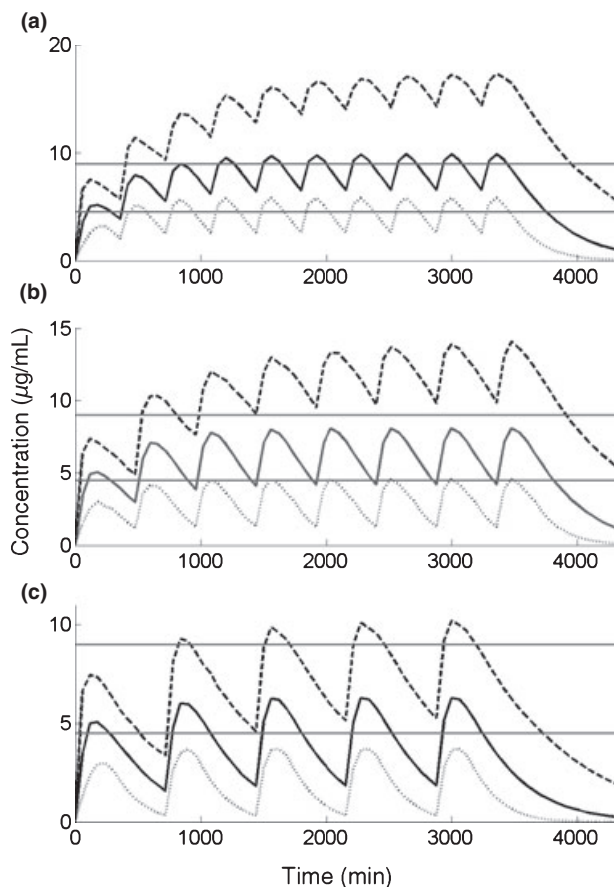
**Fig. 3.** Plots of (a) observed plasma caffeine concentration vs. population model-predicted caffeine concentration; (b) weighted residual vs. population model-predicted caffeine concentration; and (c) weighted residual vs. subject number.

### Concentration simulations

Simulated plasma caffeine concentrations for 500 non-smokers and 500 smokers, separately, after multiple oral doses of 204, 340 and 476 mg (repeated dosing every 6, 8 or 12 h) were assessed in conjunction with the reported range of 4.5–9  $\mu\text{g/mL}$  within which caffeine has beneficial effects (12, 13). For the non-smokers, the results showed that the peak and trough 50th percentile caffeine concentrations predicted under a dosing regimen of 340 mg repeated every 8 h were largely within the beneficial range (Fig. 4). In contrast, for the smokers, the peak and trough 50th percentile caffeine concentrations predicted under a dosing regimen of 476 mg repeated every 6 h fell within the beneficial range (Fig. 5).

### DISCUSSION

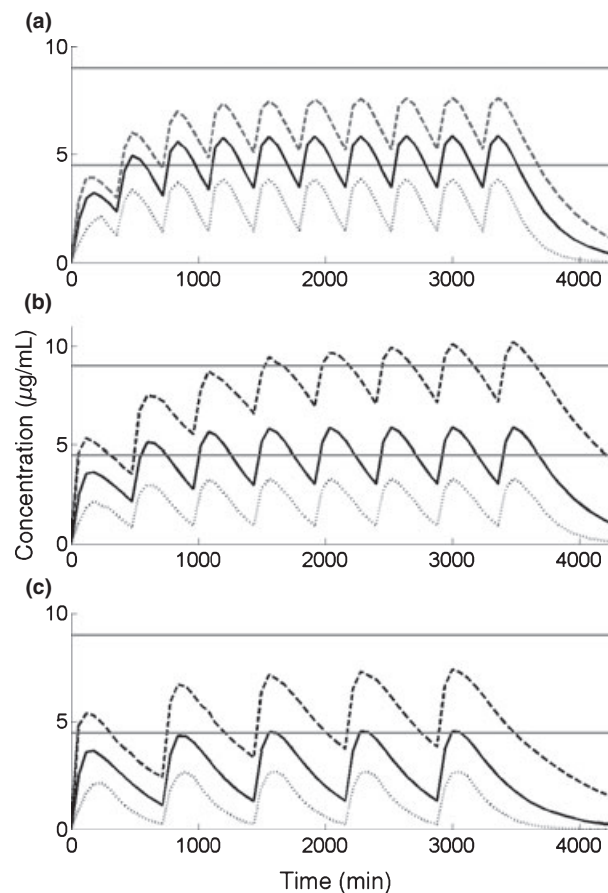
To our knowledge, this is the first investigation in which population pharmacokinetic modelling of oral caffeine has been applied to young healthy



**Fig. 4.** Predicted 50th percentile caffeine concentrations for the 6- (a), 8- (b) and 12-h (c) interval repeated dosing regimens at 204 (dot line), 340 (continuous line) and 476 mg (dash line), plotted against time for the non-smokers. The 5th and 95th percentile predictions are not shown for clarity purposes. The plots were obtained by simulating 500 smokers each with the present study's median weight of 68 kg. In each subplot, the lower and upper horizontal continuous lines indicate the lower (4.5 µg/mL) and upper (9 µg/mL) limits, respectively, of the purported beneficial window for plasma caffeine concentration. Based on the predictions, a dosing regimen of 340 mg repeated every 8 h (continuous line in subplot (b)) appears to be the most optimal for non-smokers.

Asian adults who displayed different levels of caffeine habituation in the form of regular consumption of caffeine-containing beverages or cigarettes. A one-compartment model with first-order absorption and first-order elimination was found to best characterize the plasma caffeine concentration data.

Our mean individual estimates for  $CL/F$ ,  $V/F$  and  $K_a$  were in good agreement with estimates



**Fig. 5.** Predicted 50th percentile caffeine concentrations for the 6- (a), 8- (b) and 12-h (c) interval repeated dosing regimens at 204 (dot line), 340 (continuous line) and 476 mg (dash line), plotted against time for the smokers. The 5th and 95th percentile predictions are not shown for clarity purposes. The plots were obtained by simulating 500 smokers each with the present study's median weight of 68 kg. In each subplot, the lower and upper horizontal continuous lines indicate the lower (4.5 µg/mL) and upper (9 µg/mL) limits, respectively, of the purported beneficial window for plasma caffeine concentration. Based on the predictions, a dosing regimen of 476 mg repeated every 6 h (dash line in subplot (a)) appears to be the most optimal for smokers.

previously reported for mixed-effects modelling of plasma caffeine concentrations (115–216 mL/min, 30.84–46.46 L and 0.0102–0.096 per millilitre for  $CL/F$ ,  $V/F$  and  $K_a$  respectively) (15). Our estimated mean values of  $CL/F$  and  $V/F$  also compared favourably with the mean values reported in two previous studies, which were based on non-compartmental analysis of plasma caffeine concentrations in adults (28–200 mL/min and 17–55.09 L for



CL/F and V/F respectively) (7, 8, 16). Consistent with the results from these non-compartmental analyses, we found statistically significant differences in the values of CL/F but not in the values of V/F between the non-smokers and the smokers.

The pharmacokinetic evaluation in our population did not replicate the weight-dependency of caffeine pharmacokinetics in CL/F and V/F that was reported in neonates (17, 18). One possible explanation for this discrepancy is perhaps the higher level of variation in children – in large part because of developmental changes – in their first few weeks of life as compared to young adults. Our results showed that caffeinated-beverage consumption as a covariate did not influence the plasma caffeine pharmacokinetics. The mechanism of this effect is not clear but might indicate that daily drinking of coffee or tea did not significantly affect plasma caffeine concentrations in our study participants. This lack of an association between plasma caffeine concentration and habitual intake of caffeinated beverages has also been reported in (19).

The categorical variable cigarette smoking was a significant covariate in the final population model. This observation coupled with significantly larger mean CL for the CS group (under non-compartmental modelling) reaffirm the fact that cigarette smoking plays an important role in altering caffeine disposition in adults, primarily by increasing the inducibility of cytochrome P450 1A2, which is the main enzyme involved in caffeine metabolism (7, 8). This influence by cigarette smoking notwithstanding, it has been shown that about 72.5% of the variability in CYP1A2 activity is genetically determined (20). Additionally, sequencing of genomic DNA has revealed putative polymorphisms in exons 2 and 7 and in intron 1 of CYP1A2 (21–23). Also, the intron 1 polymorphism appears to affect the inducibility of CYP1A2. Therefore, future studies could investigate the influence of gene polymorphisms of this hepatic enzyme on inter-individual variability in caffeine pharmacokinetics of non-smokers and smokers.

Differences in hepatic drug metabolism have been commonly cited as a factor influencing the pharmacokinetics of drugs in various racial groups (24, 25). Evidence suggests that CYP1A2 activity in Orientals is lower than in Caucasians (25). A trimodal distribution in CYP1A2 activity has been

suggested for the Caucasian population, whereas a bimodal distribution may exist for Japanese (24). Our study indicates that the observed Asian caffeine pharmacokinetics and the estimated parameters' variability are comparable to those previously described, which focused primarily on the Caucasian population (Table 4). While the average values of the non-compartmental pharmacokinetic parameters differ between the Asian and Caucasian populations, there were no apparent differences after normalization for dose and body weight (data not shown). This suggests that the caffeine pharmacokinetics in Asian and Caucasian subjects are not meaningfully different.

Our final population parameter estimates were in good agreement with a previous report describing appreciable interindividual variability in young adults after oral administration of caffeine with an IIV of 33% for CL/F, 15% for V/F and 51% for  $K_a$ . Sources of variability could be anthropometry, differences in bioavailability and hepatic metabolism. The variance model supported estimation of the IOV in CL/F (27%), V/F (10%) and  $K_a$  (23%). We also noted that the IOV's for CL/F, V/F and  $K_a$  were estimated to be smaller than their IIV counterparts (27% vs. 33% for CL/F, 10% vs. 15% for V/F and 23% vs. 51% for  $K_a$ ), which according to (30) supported the present model's usage for customization of oral drug dosage regimens in a clinical setting.

Using the reported beneficial window of 4.5–9 µg/mL as the appropriate clinical target, plasma caffeine concentration-time profiles under varying multiple dosing regimens were generated in non-smokers and smokers with the population mean parameter estimates and interindividual variability. This was performed to determine appropriate dose titration protocols for non-smokers and smokers over a hypothetical period of 3 days. Our results showed that an oral caffeine dosage regimen of 340 mg repeated every 8 h was the most optimal in achieving a 50th percentile profile that fell within the beneficial range over 72 h in the non-smokers. As anticipated, owing to the increased caffeine clearance in smokers, a higher and more frequent dosage regimen (476 mg repeated every 6 h) was required to achieve a predicted median profile that fell within the beneficial range over the same time period in the smokers. This pattern of higher-amount-and-more-frequent repeated

**Table 4.** Previous pharmacokinetic studies

Study design	Caffeine
Newton <i>et al.</i> (26) Age: 21–36 years <i>n</i> = 6 non-smokers Weight = 54–84 kg	<p>Dose = 50 mg <math>t_{1/2}</math>: 5.7 h <math>CL</math>: <math>1.12 \pm 0.3</math> mL/min/kg <math>V_{ss}</math>: <math>0.35 \pm 0.1</math> L/kg</p> <p>Dose = 300 mg <math>t_{1/2}</math>: 6 h <math>CL</math>: <math>0.98 \pm 0.34</math> mL/min/kg <math>V_{ss}</math>: <math>0.52 \pm 0.07</math> L/kg</p> <p>Dose = 500 mg <math>t_{1/2}</math>: 5.7 h <math>CL</math>: <math>0.75 \pm 0.19</math> mL/min/kg <math>V_{ss}</math>: <math>0.44 \pm 0.12</math> L/kg</p> <p>Dose = 750 mg <math>t_{1/2}</math>: 7.5 h <math>CL</math>: <math>1.09 \pm 0.56</math> mL/min/kg <math>V_{ss}</math>: <math>0.64 \pm 0.14</math> L/kg</p>
Blanchard <i>et al.</i> (27) Age: 21.8 $\pm$ 1.1 years <i>n</i> = 10 non-smokers Weight = 79.5 $\pm$ 3.9 kg	<p>Dose = 393.3 <math>\pm</math> 20.2 mg <math>t_{1/2}</math>: 2.7–9.9 h AUC: 69 <math>\pm</math> 9.2 h <math>\mu</math>g/mL</p>
Lelo <i>et al.</i> (28) Age: 19–21 years <i>n</i> = 6 non-smokers Weight = 62–104 kg	<p>Dose = 270 mg <math>t_{1/2}</math>: 4.1 h <math>CL</math>: 1.07 mL/min/kg <math>V_{ss}</math>: 1.06 L/kg</p>
Joeres <i>et al.</i> (8) Age: 47 $\pm$ 18 years Weight = 78 $\pm$ 8 kg	<p>Dose = 366 mg <i>N</i> = 8 smokers <math>t_{1/2}</math>: 4.77 <math>\pm</math> 1.27 h <math>CL</math>: <math>1.43 \pm 0.54</math> mL/min/kg <math>V_{ss}</math>: <math>0.51 \pm 0.13</math> L/kg</p> <p>Dose = 366 mg <i>N</i> = 15 non-smokers <math>t_{1/2}</math>: 5.6 <math>\pm</math> 2.23 h <math>CL</math>: <math>0.96 \pm 0.35</math> mL/min/kg <math>V_{ss}</math>: <math>0.41 \pm 0.07</math> L/kg</p>
Kaplan <i>et al.</i> (29) Age: 23–41 years <i>n</i> = 2 smokers <i>n</i> = 10 non-smokers Weight = 54.5–86.4 kg	<p>Dose = 250 mg <math>t_{1/2}</math>: 3.94 <math>\pm</math> 0.5 h <math>CL</math>: <math>2.07 \pm 0.4</math> mL/min/kg <math>V_{ss}</math>: <math>0.58 \pm 0.03</math> L/kg</p> <p>Dose = 500 mg <math>t_{1/2}</math>: 4.74 <math>\pm</math> 0.6 h <math>CL</math>: <math>1.64 \pm 0.3</math> mL/min/kg <math>V_{ss}</math>: <math>0.53 \pm 0.03</math> L/kg</p>

$t_{1/2}$ , elimination half-life;  $CL$ , clearance;  $V_{ss}$ , steady-state volume of distribution.

dosing regimen for the non-smokers as opposed to the smokers is expected to be present for other time periods during which caffeine is to be administered to maintain or improve the performance quality in individuals.

There are several limitations in this study. First of all, our analysis considered only caffeine consumption via cigarette smoking, coffee or tea, which did not represent the full spectrum of possible caffeine-containing foods and beverages. Furthermore, we did not study the influence of caffeine withdrawal on caffeine pharmacokinetics in habitual users. We also arbitrarily selected a 'cut-off' value of 200 mg of caffeinated beverages per day to divide non-caffeine from regular caffeine consumers based on our internal consumption surveys. In addition, our study only examined the effects of two single doses. Consequently, the dosage simulations, which were based on multiple dosing regimens and included higher dosages than used in the study, would need prospective validation. Finally, we did not measure and evaluate the concentrations of caffeine metabolites, e.g. xanthine and uracil derivatives, because we were primarily interested in quantifying the pharmacokinetics of caffeine in the present investigation. We have left this combined pharmacokinetic analysis for a follow-up study.

In conclusion, the population pharmacokinetics of caffeine in young healthy adults were derived from oral data obtained in a clinical setting. Our finding that cigarette smoking affects  $CL/F$  reaffirms that this covariate plays an important role in caffeine disposition and elimination, and consequently influences oral caffeine dosing in Asian non-smokers and smokers. Pending the availability of more pharmacokinetic data, especially with regard to the establishment of a 'therapeutic' window, we are cautious about extrapolating any proposed dosage regimen in the non-smokers and smokers to the wider population with different sociodemographics (e.g. ethnicity, sex, lifestyle factors).

#### ACKNOWLEDGEMENTS

We thank all the test volunteers who participated in this study and for providing blood samples. We thank Li-Li Tan and Sook-Lan Tan for their excellent technical assistance. Financial support for the

study was provided by the Singapore Ministry of Defence.

#### REFERENCES

1. Smit HJ, Rogers PJ (2000) Effects of low doses of caffeine on cognitive performance, mood, and thirst in low and higher caffeine consumers. *Psychopharmacology*, **152**, 167–173.
2. Brice CF, Smith AP (2002) Effects of caffeine on mood and performance: a study of realistic consumption. *Psychopharmacology*, **164**, 188–192.
3. Van Dongen HP, Price NJ, Mullington JM, Szuba MP, Kapoor SC, Dinges DF (2001) Caffeine eliminates psychomotor vigilance deficits from sleep inertia. *Sleep*, **24**, 813–819.
4. Boutrel B, Koob GF (2004) What keeps us awake: the neuropharmacology of stimulants and wakefulness-promoting medications. *Sleep*, **27**, 1181–1194.
5. Lieberman HR, Tharion WJ, Shukitt-Hale B, Speckman KL, Tulley R (2002) Effects of caffeine, sleep loss and stress on cognitive performance and mood during U.S. Navy SEAL training. *Psychopharmacology*, **164**, 250–261.
6. Biggs SN, Smith A, Dorrian J, Reid K, Dawson D, van den Heuvel C, Baulk S (2007) Perception of simulated driving performance after sleep restriction and caffeine. *Journal of Psychosomatic Research*, **63**, 573–577.
7. Parsons WD, Neims AH (1978) Effect of smoking on caffeine clearance. *Clinical Pharmacology and Therapeutics*, **24**, 40–45.
8. Joeres R, Klinker H, Heusler H, Epping J, Zilly W, Richter E (1988) Influence of smoking on caffeine elimination in healthy volunteers and in patients with alcoholic liver cirrhosis. *Hepatology*, **8**, 575–579.
9. Barone JJ, Roberts HR (1996) Caffeine consumption. *Food and Chemical Toxicology*, **34**, 119–129.
10. Beal SL (2001) Ways to fit a PK model with some data below the quantification limit. *Journal of Pharmacokinetics and Pharmacodynamics*, **28**, 481–504.
11. Ette EI, Williams PJ, Lane JR (2004) Population pharmacokinetics III: design, analysis, and application of population pharmacokinetic studies. *Annals of Pharmacotherapy*, **38**, 2136–2144.
12. Yu G, Maskray V, Jackson SH, Swift CG, Tiplady B (1991) A comparison of the central nervous system effects of caffeine and theophylline in elderly subjects. *British Journal of Clinical Pharmacology*, **32**, 341–345.
13. Bryant CA, Farmer A, Tiplady B, Keating J, Sherwood R, Swift CG, Jackson SH (1998) Psychomotor performance: investigating the dose-response

- relationship for caffeine and theophylline in elderly volunteers. *European Journal of Clinical Pharmacology*, **54**, 309–313.
14. Graham TE, Spriet LL (1995) Metabolic, catecholamine, and exercise performance responses to various doses of caffeine. *Journal of Applied Physiology*, **78**, 867–874.
  15. Csajka C, Haller CA, Benowitz NL, Verotta D (2005) Mechanistic pharmacokinetic modeling of ephedrine, norephedrine and caffeine in healthy subjects. *British Journal of Clinical Pharmacology*, **59**, 335–345.
  16. Parsons WD, Neims AH (1982) Effects of cimetidine on caffeine disposition in smokers and nonsmokers. *Clinical Pharmacology and Therapeutics*, **31**, 656–661.
  17. Thomson AH, Kerr S, Wright S (1996) Population pharmacokinetics of caffeine in neonates and young infants. *Therapeutic Drug Monitoring*, **18**, 245–253.
  18. Falcão AC, Fernández de Gatta MM, Delgado Iribarnegaray MF, Santos Buelga D, García MJ, Domínguez-Gil A, Lanao JM (1997) Population pharmacokinetics of caffeine in premature neonates. *European Journal of Clinical Pharmacology*, **52**, 211–217.
  19. de Leon J, Diaz FJ, Rogers T *et al.* (2003) A pilot study of plasma caffeine concentrations in a US sample of smoker and nonsmoker volunteers. *Progress in Neuro-psychopharmacology and Biological Psychiatry*, **27**, 165–171.
  20. Rasmussen BB, Brix TH, Kyvik KO, Brosen K (2002) The interindividual differences in the 3-demethylation of caffeine alias CYP1A2 is determined by both genetic and environmental factors. *Pharmacogenetics*, **12**, 473–478.
  21. Nakajima M, Yokoi T, Mizutani M, Shin S, Kadlubar FF, Kamataki T (1994) Phenotyping of CYP1A2 in Japanese population by analysis of caffeine urinary metabolites: absence of mutation prescribing the phenotype in the CYP1A2 gene. *Cancer Epidemiology, Biomarkers & Prevention*, **3**, 413–421.
  22. Yokoi T, Sawada M, Kamataki T (1995) Polymorphic drug metabolism: studies with recombinant Chinese hamster cells and analyses in human populations. *Pharmacogenetics*, **5**, S65–S69.
  23. MacLeod SL, Tang Y-M, Yokoi T, Kamataki T, Dublin S, Lawson B (1998) The role of recently discovered genetic polymorphism in the regulation of the human CYP1A2 gene. *Proceedings of American Association of Cancer Research*, **39**, 396.
  24. Hasler JA, Estabrook R, Murray M *et al.* (1999) Human cytochromes P450. *Molecular Aspects of Medicine*, **20**, 1–137.
  25. Björnsson TD, Wagner JA, Donahue SR *et al.* (2003) A review and assessment of potential sources of ethnic differences in drug responsiveness. *Journal of Clinical Pharmacology*, **43**, 943–967.
  26. Newton R, Broughton LJ, Lind MJ, Morrison PI, Rogers HJ, Bradbrook ID (1981) Plasma and salivary pharmacokinetics of caffeine in man. *European Journal of Clinical Pharmacology*, **21**, 45–22.
  27. Blanchard J, Sawers SJA (1983) The absolute bioavailability of caffeine in man. *European Journal of Clinical Pharmacology*, **24**, 93–98.
  28. Lelo A, Birkett DJ, Robson RA, Miners JO (1986) Comparative pharmacokinetics of caffeine and its primary demethylated metabolites paraxanthine, theobromine and theophylline in man. *British Journal of Clinical Pharmacology*, **22**, 177–182.
  29. Kaplan GB, Greenblatt DJ, Ehrenberg BL, Goddard JE, Cotreau MM, Harmatz S, Shader RI (1997) Dose-dependent pharmacokinetics and psychomotor effects of caffeine in humans. *Journal of Clinical Pharmacology*, **37**, 693–703.
  30. Schiltmeyer B, Klingebiel T, Schwab M *et al.* (2003) Population pharmacokinetics of oral busulfan in children. *Cancer Chemotherapy and Pharmacology*, **52**, 209–216.