CHAPTER 6

Caffeine pharmacokinetics and effects on central and autonomous nervous system parameters in adolescents

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ABSTRACT

Despite the highly prevalent use of caffeine among adolescents, remarkably little research has been conducted regarding the physiological and behavioral effects of caffeine in this age group. Data obtained from animal studies suggest that the effects of caffeine reported in adults cannot be extrapolated simply to adolescents. Therefore, we evaluated the effect profile of caffeine on central and autonomic nervous system parameters following the consumption of a low dose caffeinated beverage by healthy adolescents; the results were compared with data obtained following the consumption of a non-caffeinated beverage. Caffeine concentrations were measured from saliva samples. In a separate study using adult volunteers, we determined the extent of oral contamination with caffeine after consuming a caffeinated beverage versus swallowing a caffeine capsule. In addition, because previous studies in adults correlated caffeine's effects with changes in plasma concentration (but not saliva concentration), both saliva and plasma samples were collected simultaneously in order to measure the saliva-to- plasma (S/P) ratio of caffeine concentration. Based on the data collected from this kinetic study, a population PK model was built to estimate plasma drug levels in adolescents; this model could prove useful to develop a PK/PD model. In adolescents, caffeine had significant effects on task parameters related to attention and visuomotor coordination (adaptive tracking task) and alertness (saccadic peak velocity). The cognitive effects of caffeine included an increase in error rate in the attention switch task. Plasma caffeine concentrations in adults were described best as a two-compartment model with a dose depot, first-order absorption kinetics, and first-order elimination kinetics. Lean body mass-dependent variability was identified for the volume of the central compartment. This PK model was expanded to a population model that described saliva caffeine concentrations in adults >1 hour after administration as a fraction (0.68) of plasma concentration. Caffeine's early effects in adolescents (i.e., within one hour) were not suitable for inclusion in a PK/PD model. In conclusion, in healthy, alert adolescents, low-dose caffeine has significant effects on parameters regarding alertness and reaction speed. Whether these effects observed in adolescents are larger in adolescents than in adults remains to be determined.

Introduction

Caffeine (1,3,7-trimethylxanthine) is the most commonly used psychoactive substance worldwide[1]; caffeine is widely available in foods, dietary supplements, chewing gum, beverages, and many over-the-counter combination analgesics. Many adolescents use caffeine as a way to enhance their academic or athletic performance[2] and to intentionally postpone sleep during nighttime leisure activities[3]. Given that some caffeine-containing products are marketed directly to children and adolescents, and given that caffeine use among children and adolescents has increased by 70% since 1977[4], it is important to understand better the potential effects – both positive and negative – of caffeine use within this particular population[2]. An extensive body of research regarding the behavioral and psychomotor effects of caffeine in adults shows that caffeine produces behavioral effects that are similar to the effects of 'classic' central nervous system (CNS) stimulants such as cocaine and amphetamine. However, despite its high incidence of use among adolescents, remarkably little research has been conducted regarding the physiological and behavioral effects of caffeine in this specific age group[2,5].

Caffeine's effects in adolescents can differ from its effect in adults[2]. To date, no studies have compared the effects of caffeine between adolescents and adults. However, prepubescent children experience more objective effects of caffeine than adults at doses of 3-10 mg/kg; these effects include increased motor activity, increased speech rate, and decreased reaction time. After ingesting caffeine, adults generally report side effects that children do not appear to experience; however, autonomic measures of arousal are affected similarly in both age groups[6]. A relatively limited number of animal studies regarding the correlation between age and caffeine's effects have shown that caffeine-induced locomotor stimulation is higher in adolescent rats than in adult rats[7], and adolescent rats may respond more robustly than adult rats to adaptive changes associated with chronic caffeine consumption[8]. These age-dependent effects are likely not limited to caffeine, as age-dependent effects have been reported for other CNS stimulants – including amphetamine[9], cocaine[10,11], and methylphenidate[12,13] – potentially due to the functional inhibition of dopamine D_1 receptors at young age[11]. Based on these findings, the effects of caffeine that have been reported in adults likely cannot be extrapolated simply to adolescents.

Here, we measured the effect profile of caffeine on both central and autonomic nervous system parameters after consuming two cups of espresso by adolescents who usually infrequently consume caffeine. Saliva samples were collected in order to measure caffeine concentration. Because the saliva can be contaminated by residual caffeine after drinking a caffeinated beverage, we performed a second study in young adult volunteers to compare the extent of contamination between drinking a caffeinated beverage and swallowing a caffeine capsule. In addition, although caffeine's effects have been correlated to changes in plasma caffeine concentrations[14,15], the relationship between caffeine's effect and saliva concentrations has not been studied. Therefore, we collected saliva and plasma samples simultaneously in order to measure the saliva-to-plasma (S/P) ratio for caffeine concentration. Based on the data obtained from this kinetic study, a population pharmacokinetic (PK) model was developed to estimate plasma drug levels in adolescents, and this model could potentially be used to develop a PK/PD model.

Methods

Subjects

ADOLESCENT STUDY

Healthy male and female adolescents (15-18 years of age) were included. The subjects were non-smokers and consumed ≤14 units of alcohol per week. After providing written informed consent (plus consent from a parent or legal guardian for subjects <18 years of age), the subjects were medically screened within three weeks of the start of the study; subjects who presented with relevant clinical abnormalities were excluded. We also excluded subjects who used

medications and/or agents known to affect caffeine metabolism and/or CNS performance; urine drug screens were performed during the selection process and prior to each test day. The study was approved by the Central Committee on Research involving Human Subjects.

KINETIC ADULT STUDY

The inclusion criteria for this study included healthy male or female volunteers 18-35 years of age with a body mass index of 18-30 kg/m^2 and body weight of 50-90 kg. Exclusion criteria included a personal history of impaired physical or mental health; a history of drug, substance, and/or alcohol abuse; and abnormal findings with respect to medical history, physical examination, ECG, vital signs, and/or blood and urine laboratory results. The subjects were instructed not to use any medications, dietary supplements, or food products that would potentially affect the metabolism of caffeine within one week of the start of the study. The subjects were instructed not to consume more than five units of xanthine-containing products, to smoke more than five cigarettes per day or to consume more than 21 (male subjects) or 14 (female subjects) units of alcohol per week. The subjects had to refrain from consuming xanthine-containing products (within days of caffeine administration and throughout the study day) or alcohol and cigarettes (within 12 hours of caffeine administration and throughout the study day). On study days, the subject's use of medications, alcohol, and/or drugs was questioned, and a urine drug screen, pregnancy test (where applicable), and alcohol breath test were performed prior to the start of any study-related procedures. This study was approved by the Medical Ethics Committee of the Leiden University Medical Center, Leiden, the Netherlands.

Study design

ADOLESCENT STUDY

This study was a randomized, double-blind, placebo-controlled, two-way crossover study. Each subject received two cups of espresso coffee containing approximately 135 mg caffeine (according to the manufacturer) or two cups of decaffeinated espresso coffee containing <10 mg caffeine on two separate study days. To increase palatability, sucrose and dehydrated milk were available. This dose of caffeine (135 mg) was expected to block adenosine receptors and – to a small extent – phosphodiesterases[16], while minimizing undesired adverse events. Decaffeinated coffee was chosen as a placebo to make the two intervention arms as comparable as possible with respect to expectancy, sensory effects (due to the similar taste of caffeinated and decaffeinated coffee) and the presence of biologically active substances other than caffeine[17]. The beverages were administered following a minimum fasting period of four hours. A standardized light breakfast and a standardized lunch were offered 2.5 hours and 4.5 hours after coffee consumption, respectively. Water was provided ad libitum. The subjects were confined to the clinical research unit for approximately six hours after caffeine consumption.

Based on previous studies conducted at CHDR, a sample size of 16 subjects was sufficient to detect a 10% in adaptive tracking and a 5% change in saccadic peak velocity with 80% power (two-sided test, alpha = 0.05) using a randomized Williams square design.

KINETIC ADULT STUDY

This study was a randomized, open-label, two-way crossover study. Each subject received two cups of espresso coffee containing approximately 135 mg caffeine or one capsule containing 200 mg coffeine (Pharmaline, Oldenzaal, the Netherlands) on two separate study days. The dose of 200 mg in capsule form was chosen based on a PK model using data from the study in healthy adolescents and reported data in adults (Figure 1). The capsule was administered with approximately two cups of water. Where applicable, female subjects were studied while taking their oral contraceptive (i.e., not during the stop week). The caffeinated beverage or capsule was administered following a minimum fasting period of four hours. A standardized light breakfast, lunch and dinner were offered 2 hours, 4^2/3 hours and 9 hours after caffeine administration. Water (200 ml) was given every two hours after the caffeine

administration to maintain all subjects on a consistent hydration schedule. The subjects were confined to the clinical research unit for approximately 11 hours after caffeine administration.

For this study, no formal power calculation was performed. However, based on previous experience, we expected that a sample size of six subjects would be sufficient for determining the pharmacokinetic parameters and the saliva-to-plasma ratios.

In both the adolescent and adult studies, the wash-out period between study days was ≥3 days, and caffeine (or placebo) was administered at the same time of day to minimize any confounding effect of circadian rhythm.

Pharmacokinetics

In adolescents, saliva was collected in Salivette tubes (Sarstedt, Numbrecht, Germany) prior to caffeine administration (to confirm compliance) and every 15 minutes thereafter for 120 minutes, then every 60 minutes until the 240-minute time point, then every two hours until the 360-minute time point. In the adult study, the post-caffeine sampling time points were determined based on a PK model using data from the study in healthy adolescents and reported data in adults (see Figure 1); samples were collected prior to caffeine administration, 10, 30, and 60 minutes after administration, and 2, 4, 7, and 11 hours after administration. Because caffeine saliva PK can be complicated by pH partitioning, saliva was collected actively, as stimulated saliva flow leads to a stronger correlation between plasma and saliva caffeine levels[18,19]. At each time point, the subjects were instructed to insert three Salivette in their mouth and move it around the oral cavity for two minutes. Both saliva pH and saliva flow rate were measured to determine whether any fluctuation in these parameters might account for any remaining variability in the S/P caffeine ratio. Immediately after collecting and weighing the saliva (to measure salivary flow), the swabs were centrifuged at 2000x*g* for 10 minutes at 4 °C. The three saliva samples per subject were then pooled, and pH was measured using a Symphony pH meter (model SP70P, VWR Scientific) equipped with a pH/Redox electrode (range: -2.000 to 19.999; relative accuracy: ±0.002). The saliva was then transferred to 2-ml tubes (Sarstedt) and immediately stored at -80°C.

For PK analyses, venous blood samples were obtained from the adult subjects via an indwelling catheter four minutes after the start of each saliva sample collection. Blood samples were collected in 6-ml EDTA tubes and kept on ice. The samples were centrifuged (2000x*g* for 10 minutes at 4°C) as soon as possible after collection, but within 30 minutes of collection. The saliva supernatants and plasma samples were stored at -20°C until analysis.

Caffeine concentrations in the saliva samples obtained during the adolescent study were measured at Erasmus Medical Center (Rotterdam, the Netherlands). Caffeine concentrations in the saliva and plasma samples obtained during the adult study were measured at the Academic Medical Center (Amsterdam, the Netherlands). In both laboratories, a validated high-performance liquid chromatography method was used[19]. Samples were either analyzed immediately upon arrival at the laboratory or stored at 4°C until analysis (within 24 hours). The limit of quantification was 0.2 mg/L in a sample volume of 100 µl.

Pharmacodynamics

An extensive CNS battery was implemented to determine which functional CNS domains are affected by caffeine. All pharmacodynamics (PD) measurements—with exception of the visual and verbal learning task (VVLT)—were performed at the same time points as the saliva collection time points (see above). VVLT was administered approximately 45 and 150 minutes after caffeine administration in order to assess immediate and delayed recall. The PD measurements were performed in a quiet room with ambient lighting, with only one subject in the room per session. Prior to the first study day, the subjects were familiarized with the experimental procedure and performed a practice testing session to minimize potential learning effects during the study days. The tests were performed as described below.

SACCADIC AND SMOOTH PURSUIT EYE MOVEMENT

Saccadic and smooth pursuit eye movements were recorded as described previously[20–22]; in adults, these movements can be affected by many drugs that act upon the CNS, including GABAergic[23], serotonergic[24], noradrenergic[25,26], and dopaminergic drugs. For this test, we use a computer-based system for data recording and analysis (Cambridge Electronics Design, Cambridge, UK), a Nihon Kohden device for stimulus display, signal collection, and signal amplification (Nihon Kohden Corporation, Tokyo, Japan), and single-use surface electrodes (Medicotest N-00-S, Olstykke, Denmark). The average values for latency (i.e., response time), peak saccadic velocity, and the inaccuracy of all artifact-free saccades were used as parameters for quantifying saccadic eye movements. Saccadic inaccuracy was calculated as the absolute difference between the stimulus angle and the corresponding saccade, expressed as a percentage of the stimulus angle. A higher percentage reflects poorer performance on the eye movement test. For smooth pursuit, the target moved in a sinusoidal pattern over 20 degrees of eyeball rotation at a frequency ranging from 0.3–1.1 Hz. The primary parameter was the percentage of time that the eyes pursued the target smoothly.

BODY SWAY

The body sway metric records body movements in a single plane, thus providing a measure of postural stability. A variety of CNS-active drugs induce a change in body sway, including GABAergic drugs[27,28], cannabinoids such as tetrahydrocannabinol[29], and CNS stimulants such as methylphenidate[30,31]. Body sway was measured as described previously[32].

ADAPTIVE TRACKING

Adaptive tracking measures visuomotor coordination and vigilance and was performed as originally described by Borland and Nicolson[33]. This test was adapted for use on a personal computer. The adaptive tracking test has been used previously to measure the CNS effects of alcohol, sleep deprivation, and a wide range of CNS-active drugs, including stimulants such as methylphenidate[30,31]. The average performance during a 3-minute testing period was used for statistical analysis.

LEFT/RIGHT DISTRACTION TASK

A parametric version of the color-word response conflict task[34] was used to measure intervention-induced inhibition[35]. This task has been used previously to measure the effects of several compounds, including antipsychotics[36]. In this task, the word 'Left' or 'Right' was displayed on either the left or right side of a computer screen. The subject was instructed to respond quickly by pressing the button corresponding to the location of the word, irrespective of the word's meaning. The output parameters included response time and response accuracy as a function of task difficulty.

FINGER TAPPING

The finger tapping test evaluates motor activation and fluency and was adapted from the Halstead-Reitan Neuropsychological Battery[37]. During the test, the rate of tapping the index finger on the dominant hand was measured; a session comprised five 10-second tests. The subject was instructed to tap the index finger as rapidly as possible on the space key of a computer keyboard. The output measure was mean tapping rate and standard deviations were used for statistical analysis.

VISUAL AND VERBAL LEARNING TASK

The VVLT has been used previously to identify the CNS effects of various compounds, including benzodiazepines[38], antidopaminergic agents[32], and cannabinoid drugs[29]. This task was performed as described previously[32]. The primary outcomes for the immediate and delayed word-recall tasks were the numbers of correct responses; the primary outcomes for the delayed word recognition task were the number of correct items and the mean response time for correct responses. Learning was measured using the change in reproduced words with three consecutive memorization trials, decay from the change in reproduced words after a time delay (delayed word recall versus the last trial

of immediate word recall), and retrieval (the difference between delayed word recognition and delayed word recall).

ATTENTION SWITCHING TASK

This task was used to measure executive control. CHDR uses an adaptive version of a task-switching design with a Go/no Go task as described by Wylie and colleagues (2003). The output parameters are response time and error-rate.

PUPIL SIZE MEASUREMENT

Measurement of the pupil size was done by taking a picture from both eyes simultaneously while the subject was seated on a chair with his head fixed in the head support system. From the pictures the ratio between the pupil - and iris diameter was calculated

BLOOD PRESSURE AND HEART RATE

Blood pressure was measured using a Nihon Kohden BSM-1101K automated oscillometric monitor, a Pressmate BP 8800 (Colin), or a Dash 4000 monitor. Heart rate was measured using oscillometry.

Statistical analysis and pharmacometrics

PHARMACODYNAMICS

The PD endpoints of the adolescent study were analyzed using mixed-model analyses of variance (using the SAS PROC MIXED program). Subject, subject by treatment, and subject by time were random effects; treatment, test day, time, and treatment by time were fixed effects; and the average baseline value was a covariate. Contrasts were estimated within the overall treatment effect, and contrasts between treatments >360 min were calculated. Body sway and attention switch task variables were log-transformed, analyzed, and then back-transformed (the results are presented as percent change). The attention switch error rate was transformed by adding 1 to all data points in order to avoid log-transforming a value of 0. Because the VVLT parameters were assessed only once for each treatment arm, the raw test scores were evaluated. Data analyses included an analysis of variance with baseline correction. This study tested the following null hypothesis: 'there is no difference between caffeine and placebo'.

Pharmacometrics

DATA

Exploratory individual and summary concentration-time profiles were generated in order to identify potential outliers, to understand the influence of censoring concentrations below the limit of quantification (LOQ), and to yield information regarding the base structural model. Concentration-time curves were plotted for the plasma and saliva samples in order to identify indirect relationships (e.g., time shifts). Samples below LOQ before T_{max} were set to zero.

MODELING STRATEGY

Pharmacometrics were performed using nonlinear mixed-effect modeling (NONMEM version 7.2.0[39], Icon Development Solutions, Ellicott City, MD, USA, 2009). First-order conditional estimation with interaction (FOCEI) was used for the estimation, with a convergence criterion of five significant digits in the parameter estimations. NONMEM reports the objective function value (OFV), which is the -2 times log likelihood (-2LL). Models were compared using the likelihood ratio test with the assumption that the difference in -2LL is Chi-square distributed, with degrees of freedom determined by the number of additional parameters in the more complex model. Therefore, with a decrease in OFV of at least 6.63 points, a model with one additional parameter is considered superior to its parent model ($p<0.01$). Different models with increasing complexity were compared to identify the simplest model that described the data adequately. Graphical analysis was used to assess model performance while developing the model.

The following goodness-of-fit plots were prepared: observed concentration (the dependent variable, or DV) versus population concentration (PRED); DV versus individual predicted concentration (IPRED); weighted residuals with interaction (CWRESI) versus IPRED; CWRESI versus time after dose; combined PRED, IPRED, and DV versus time (per individual) and distribution of inter-individual variability (IIV) estimates (ETA). Covariates were analyzed using a stepwise approach. For the visual predictive check (VPC), dosing regimens were simulated as performed in the adolescent and adult studies based on the distribution of lean body mass (LBM[40]) in the data sets. The best PK and PK/PD models were selected based on the likelihood ratio test, diagnostic plots, VPC, parameters, and precision in parameter estimates. The relative standard error (RSE) was calculated and used to derive the uncertainty in the parameter estimates; RSE <10% was considered acceptable. Preparing the NONMEM input file and processing of the model results (i.e., preparing tables and graphs) was performed using R version 2.12.0[41]. VPC was performed using the lsoda function from the deSolve library (version 1.8.1) and the function mvrnorm from the MASS library (version 7.3-8) by simulating 1000 replications of the best models and a simulation data set (up to 12 hours). The median prediction and 95% prediction interval were calculated for each simulated time point and compared to the observations.

POPULATION PK MODEL DEVELOPMENT

First, a population plasma PK model for caffeine was developed using the plasma data obtained from the adult study. One-compartment and two-compartment structural models, as well as different compartmental and elimination sub-models, were tested. All models used a first-order process to describe the oral absorption of caffeine. Interindividual variability (IIV) was assessed separately for each PK parameter using a stepwise bottom-up approach. Correlations between the IIV of each parameter were determined graphically. When a correlation was found to be significant (either by shape or Pearson's correlation coefficient), covariance between the terms was assessed by applying an omega block on selected parameters and accepted based on the results of a likelihood ratio test. For the parameter estimation, shrinkage <30% was considered acceptable[42]. Proportional and combined error structures were evaluated to best describe the residual error. Based on graphical identification, the most promising covariates were tested in the model; these covariates were then included based on a decrease in OFV in a stepwise manner (forward inclusion of covariates, followed by a backward elimination step). A plasma-saliva PK model for caffeine was then developed using the saliva data collected from the study in adults. All plasma PK parameters were fixed to the individual estimates that were derived from the best plasma PK model. The simulated individual plasma concentration-time profiles consequently drive the saliva caffeine concentrations, for which different model structures were explored (e.g., a linear effect model or an effect compartment). The model was developed as described for the best PK plasma model (e.g., by incorporating IIV, residual error, and covariates). The best model was validated by performing a VPC of the simulated saliva concentrations in adolescents.

Results

Subjects

A total of 16 adolescent subjects (10 males, 6 females) 15-18 years of age (mean: 16.8 years) were enrolled in 2009, and a total of 7 adult subjects (5 males, 2 females) 19-24 years of age (mean: 21.4 years) were enrolled in 2012. All adolescent subjects were attending pre-university secondary education (in Dutch, *Voortgezet Wetenschappelijk Onderwijs* or VWO). One of the male adult volunteers discontinued the study after the first study day (a caffeine capsule) for reasons unrelated to the study and was replaced. The mean weight and height of the adolescent subjects were 69 kg (range: 50-85 kg) and 1.80 m (range: 1.65-1.93 m), respectively; the mean weight and height of the adult subjects were 77 kg (range: 58-90 kg) and 1.82 m (range: 1.70-1.98 m), respectively. None of the adolescents reported using cigarettes; one adult subject reported smoking four cigarettes per day. One female adult subject used an oral contraceptive

during the study. In addition, one adolescent subject used paracetamol during the study (a single 500-mg dose taken 2.5 hours prior to dosing on the first study day.) The mean daily consumption of xanthine-containing products in the preceding months was 2.6 (range: 0-5) standard units for the adolescents and 1.7 (range: 0-4) standard units for the adults.

Pharmacodynamic results

NEUROPHYSIOLOGICAL PARAMETERS

Caffeine administration significantly increased adaptive tracking performance and saccadic peak velocity and accelerated saccadic reaction time (Table 1, Figure 2 and Figure 3). No significant effect was observed with respect to body sway, saccadic eye movement (response latency), and tapping. In addition, no significant effect was observed with respect to parameters related to memory (Table 2).

Attention switch task error rate and response time (pre-switch trial, #3) were both significantly affected by caffeine (Table 3, Figure 4). In the distraction task, the response times for incorrect responses were shorter than the response times for correct responses (Table 3).

Neither systolic blood pressure nor pupil size differed significantly between the caffeine and placebo groups (Table 4).

Pharmacometrics analysis

POPULATION PK PLASMA MODEL DEVELOPMENT

A total of 90 PK observations from plasma samples were collected from six adult subjects. One data point obtained prior to T_{max} was below LOQ and was set to zero.

A two-compartment model with a dose depot and first-order absorption and elimination kinetics described the caffeine plasma concentrations in the adult subjects best. The parameter estimates, relative standard error (RSE) and IIV are summarized in Tables 5 and 6. The parameter for lag time after oral administration (ALAG1) was fixed at a value of 0.23 hours (the time point before the first PK sample), as this parameter was needed in order to describe the absorption phase; however, the number of data points in the absorption phase was not sufficient to accurately quantify the two necessary absorption parameters (ka and ALAG). For the beverage treatment group, V2 (the distribution volume for the central compartment) was corrected by the fractional bioavailability, as bioavailability differs between beverage and oral treatment. To facilitate fitting of the model, the initial parameter estimates and boundaries for the parameters estimates were selected based on published values. The residual error model included combined proportional and additive errors. IIV was identified on ka (the absorption rate constant) – with different variability between the two treatments – on clearance, on ke and on the correction fraction of V2 for the beverage group. Two parameter estimates with large confidence intervals (ka and Q) were modeled as an exponent in order to prevent negative – and thus physiologically impossible – values for these parameters. LBM was implemented in the model as a covariate of V2 in normalized power function (Eq. 1).

$$P_i = P_p \times (COV/COV_{median})^k \qquad \text{Eq. 1}$$

where P_i; individual parameter estimate, P_p; typical (population) value, COV; individual covariate value, COV_{median}; normalization value for covariate, k; parameter estimate for the exponent.

In the best PK model, the population concentrations and individual predicted concentrations were close to the observed values. In addition, we observed no apparent concentration-dependent or time-dependent bias (Figures 5 and 6).

POPULATION PK SALIVA MODEL DEVELOPMENT

A total of 614 PK observations from saliva samples were obtained from 6 adult and 16 adolescent subjects. All data points collected prior to T_{max} and below LOQ were set to zero.

The adult data were described best by a PK model that described saliva concentration as a fraction of the plasma concentration. However, this model was

valid only for observations made >1 hour after the dose. Prior to the 1-hour time point, the relationship between the plasma and saliva concentrations was too complex to develop a physiologically plausible model, regardless of whether the caffeine was delivered as a beverage or capsule (Figure 7). Therefore, only saliva concentrations measured >1 hour after dosing were included in the analysis. The estimated parameter was the S/P ratio (α; 0.68). Because incorporating IIV into the estimated ratio did not improve the model, the relationship between saliva pH or flow and the S/P ratio could not be evaluated adequately. The residual error model included an additive error. The best model accurately described saliva concentrations for both capsule and beverage dosages in adults for >1 hour after dosing (Figures 8 and 9). The best model also accurately described saliva concentrations in adolescents from >1 hour after dosing, although the saliva concentrations in adolescents were slightly under-predicted (Figure 10).

Discussion

Although the behavioral and psychomotor effects of caffeine have been studied extensively[43-45], they have not been investigated in adolescents. Because caffeine can affect adolescents and adults differently[2], results obtained in adults cannot necessarily be translated to adolescents. This is the first study to examine the CNS effect profile of a single oral dose of caffeine (a caffeinated beverage) specifically in adolescents. In adolescents, caffeine had significant effects on task parameters related to attention, visuomotor coordination (adaptive tracking task), and alertness (saccadic peak velocity). Caffeine also induced cognitive effects, including an increase in error rate in the attention switch task.

Because previous studies have demonstrated that saliva is an appropriate matrix for estimating caffeine concentration in plasma[46,47], we measured caffeine concentration in saliva samples. To correlate caffeine's effects in adolescents with the estimated plasma caffeine concentration, we performed a PK study in adults and determined the S/P ratio for caffeine concentration. Plasma caffeine concentration in adults was described best as a two-compartment model with a dose depot, first-order absorption kinetics, and first-order elimination kinetics. Although some published reports suggest that caffeine PK in plasma can be described using a one-compartment model[48], some individual concentration-time profiles suggested two-compartment properties. Therefore, a two-compartment model was tested and was found to be superior to the one-compartment model. The apparent clearance and distribution volume were consistent with published values in adults[48-50] and were estimated here with high precision. For the caffeinated beverage, the dose identified by the model was lower (by a factor of 0.67) than the dose indicated by the manufacturer (135 mg) and varied among the subjects. With respect to ka, the relative standard error of the population values was high, which could be attributed—at least in part—to the inclusion of PK data obtained after the administration of different formulation types, the low number of observations collected during the absorption phase, and caffeine's moderately fast absorption rate. Two IIV terms were required for the absorption rate (one for each formulation), and an arbitrarily fixed lag time of 0.23 hours was needed in order to stabilize the model. IIV for the absorption rate was large for both formulations compared to IIV terms for other PK parameters, and this likely contributed to unexplained variability in the plasma C_{max} and T_{max} values. In addition, this high degree of variability necessitated the use of a relatively large number of parameters in order to describe the data accurately. Thus, although this population PK model adequately describes the variability in data obtained in this study population, it should be used with caution when used for predictive purposes. Consistent with previous studies in adults[50], we also observed IIV in the apparent clearance and apparent distribution volume (in the beverage group only). Additionally, IIV was identified in inter-compartmental clearance in the beverage group only (as this parameter is related to the apparent distribution volume) and the elimination rate constant. Lean body mass-dependent variability was identified for the volume of the central compartment. Other potential covariates, including smoking status[50] and

oral contraception[51], could not be evaluated, as only one subject smoked and only one female subject was taking birth control pills at the time of the study.

The population PK model for plasma caffeine was extended by including a population model that described caffeine saliva concentration in adults >1 hour after administration; the model showed that saliva concentration was a fraction (0.68) of plasma concentration, which is consistent with previous studies in neonates[19] and adults[20]. The relationship between plasma and saliva caffeine concentrations could not be described as linear at earlier time points. Several studies in healthy adults reported a time-dependent S/P ratio for caffeine concentration, with a higher initial ratio that was followed by a decrease in ratio at later time points[52-54]. This time-dependent phenomenon has been attributed to fluctuations in the arteriovenous blood concentration[54] and pH partitioning[53]. In our PK study in adults, although saliva pH and flow were measured at each sampling time point, they could not be quantified as covariates in our model.

A slight under-prediction was observed with respect to simulations of saliva concentration in adolescents. This may have been due to several factors in addition to the abovementioned limitations in the plasma PK model. Possible study-related factors include a variable—and therefore potentially slightly higher—dose in the adolescent study. Contamination also likely played a role in the early time points; however, this was likely not a factor at time points >1 hour after administration. In addition, the simulations were based on the distribution of LBM in the adolescent data set, which included more male subjects (with generally higher LBM) than female subjects, perhaps explaining why the observed saliva concentrations were in the upper half of the 95% prediction interval for the majority of female subjects. The formula that we used to calculate LBM[18] may have caused an under-estimation of LBM in some of the male adolescents. This formula uses weight and body mass index (height/weight^2) and is based on data from adults, as no validated equation has been developed for adolescents ≥15 years of age. However, in a recent study, an equation for LBM that included only height and weight resulted in the systematic under-estimation of LBM in male adolescents between 13 and 15 years of age[55]. Finally, other factors could be related to differences between adolescents and adults, although an age-dependent difference in the S/P ratio is unlikely, as reports suggest similar ratios across various ages[19,20]. Relative under-prediction of adolescent saliva concentrations may reflect differences in PK variability between adolescents and adults. A pubertal stage dependent decrease in CYP1A2-mediated caffeine clearance has been described in healthy adolescents[56], and weight-normalized clearance values of caffeine's active metabolite theophylline in sexually mature adolescents have been reported to be lower (~45%) than those in prepubescent individuals[57]. Finally, because adolescents of the same age range can have different levels of physiological maturation, caffeine clearance is likely to vary widely among adolescents, resulting in a broader 95% prediction interval (and thus also higher concentrations at the highest percentile) than previously estimated using data obtained from adults.

Because the S/P ratio for caffeine could only be described accurately >1 hour after administration, the resulting PK model was not sufficient for developing a PK/PD model using adolescent data alone. Significant effects in adolescents were seen one hour of administration as well as 3-4 hours after administration. For example, caffeine's effects on the adaptive tracking task peaked soon after administration, which may be explained by caffeine's low EC_{50} (half-maximal effective concentration). Alternatively, caffeine could facilitate task learning (learning effects) rather than task performance. Caffeine's effects that occurred within one hour were not suitable for developing a PK/PD model based on simulated plasma caffeine concentration; however, these effects could be included in a PK/PD model that is based on measured saliva caffeine concentration. With compounds that diffuse freely, including caffeine, the salivary concentration provides a better estimate of the drug's cellular concentration in organs than in peripheral venous blood[54]; thus, saliva concentrations may be superior to plasma concentrations for developing a model. However, such a model must be corrected for possible contamination (particularly in individual samples collected directly after administration of a caffeinated beverage). Unfortunately, contamination could not be estimated

in one of the sub-models due to the limited number of observations. Caffeine's effects that occur >1 hour after administration could be used to develop a PK/PD model. For example, caffeine's effects on saccadic peak velocity occurred 3-4 hours after administration, and several of caffeine's effects occur with a time delay relative to changes in plasma concentrations, with an equilibration half-life of 20-50 minutes[14,15]. However, whether these late effects can be attributed to caffeine's distribution time into tissues, post-receptor changes, or the formation of active metabolites (such as theophylline, which acts as an adenosine receptor antagonist[58]) remains unclear.

In our study of adolescents, a low to moderate dose of caffeine of approximately 135 mg was chosen because this dose can significantly block adenosine receptors[16]. However, the actual dose of caffeine that was absorbed from the beverage was likely lower than 135 mg, as the plasma model identified a dose of 90 mg in the adults who ingested the same caffeinated beverage. Despite this decreased effective dose, caffeine-related effects were observed in parameters regarding alertness and reaction time in adolescents, effects that are more commonly observer following a low to moderate dose of caffeine in adults[59,60]. It is therefore likely that larger changes would occur at higher caffeine doses for some outcome parameters such as body sway, response times (of saccadic eye movements and the distraction task), and blood pressure; parameters that changed slightly but did not reach statistical significance. For example, a dose-dependent increase in diastolic blood pressure was reported in a recent study in adolescents[61]. In contrast to previous findings in adults, caffeine's effects on parameters in the attention switch task included an increase in error rate, but no effect on response time in the switch trial. Performance in repeated-task trials is typically better than performance in 'switch' trials. Caffeine has been shown to reduce switch error rates and/or response time relative to placebo[62,63], and coffee consumption has been suggested to improve task-switching performance by enhancing anticipatory processing (e.g., task set updating), presumably via caffeine's neurochemical effects on the dopaminergic system[62]. In addition, in our study the response time for incorrect responses was shorter than the response time for correct responses in the distraction task, which may suggest that impulsivity increases after caffeine intake in adolescents. Neuroimaging studies have shown that task switching involves an extensive neural network, including lateral prefrontal and parietal cortical regions, the pre-supplementary motor area, and the anterior cingulate cortex[64-69], neural regions that functionally mature during adolescence[70]. In addition, animal studies suggest that the dopaminergic system undergoes major changes during adolescence (for review, see[71]); in addition, other CNS stimulants (for example, amphetamine) can have age-dependent effects on impulsivity[72]. However, as the switch trial is not designed to evaluate impulsivity, it remains to be determined if caffeine indeed increases impulsivity in adolescents.

In conclusion, we report that a low dose of caffeine induces significant effects on parameters regarding alertness and reaction speed in adolescents, despite an expected ceiling effect on several parameters among this healthy, alert population. Whether these low-dose effects of caffeine are stronger in adolescents than in adults remains to be determined. Caffeine did not improve task-switching performance, but it may increase impulsivity. Finally, because the S/P ratio for caffeine in adults could only be described accurately >1 hour after receiving the dose, and because the level of contamination after consuming the caffeinated beverage could not be quantified, the early effects of caffeine in adolescents were not suitable for developing a PK/PD model.

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TABLE 1 **Least-square means, estimates of difference and confidence intervals of neurophysiological effects of caffeine**

Parameter	Least-square means		Estimate of difference (95% CI)	p-value
	placebo	caffeine	caffeine vs. placebo	
Body Sway (mm)	272	248	-8.85% (-18.5/1.96%)	0.098
Saccadic Inaccuracy (%)	6.3	6.4	0.1% (-0.3/0.6%)	0.559
Saccadic peak velocity (deg/sec)	517	526	8.5% (2.0/15.0%)	**0.014**
Saccadic Reaction Time (sec)	0.197	0.193	-0.005% (-0.009/0%)	0.068
Smooth pursuit (%)	49.4	49.2	-0.3% (-3.1/2.6%)	0.842

95% CI = 95% confidence interval; statistically significant differences (i.e., p<0.05) are indicated in bold

TABLE 2 **Least-square means, estimates of difference and confidence intervals of effects of caffeine on cognition**

Parameter	Least-square means		Estimate of difference (95% CI)	p-value
	placebo	caffeine	caffeine vs. placebo	
Immediate word recall number correct (#3)	19.4	19.9	0.5 (-1.5/2.5)	0.6023
Delayed word recall number correct	17.5	18.1	0.6 (-1.9/3.0)	0.6330
Delayed word recognition number correct	27.1	26.4	-0.7 (-2.1/0.7)	0.3025
Response time of correct responses (word recognition)	880.2	847.0	-33.3 (-82.2/15.7)	0.1676

95% CI = 95% confidence interval; # = trial number

TABLE 3 **Mean reaction times and error rates of attention switch task, adaptive tracking, tapping and distraction task**

Parameter	Least-square means		Estimate of difference (95% CI)	p-value
	placebo	caffeine	caffeine vs. placebo	
Attention switch error rate (#1)	0.7	1.1	19.11% (1.61/39.62%)	**0.0338**
Attention switch reaction time (#3)	19525	17929	-8.17% (-11.0/-5.25%)	**<0.0001**
Adaptive tracking	26.55	28.06	1.50 (0.60/2.41)	**0.0031**
Tapping (taps/10s)	63.6	65.1	1.5 (-0.3/3.4)	0.0975
Left/right distraction number correct	31	31	-0 (-1/0%)	0.1137
Left/right distraction number incorrect	1	1	0 (-0/1%)	0.1137
Left/right distraction reaction time correct (ms)	17649	17036	-613 (-1261/34)	0.0611
Left/right distraction				
Reaction time incorrect (ms)	558	704	146 (-56/347)	0.1395

95% CI = 95% confidence interval; # = trial number

TABLE 4 **Least-square means, estimates of difference and confidence intervals of effects of caffeine on autonomic nervous system**

Parameter	Least-square means		Estimate of difference (95% CI)	p-value
	placebo	caffeine	caffeine vs. placebo	
Left pupil/Iris ratio	0.5640	0.5582	-0.006 (-0.020-0.0084)	0.3528
Right pupil/Iris ratio	0.5561	0.5598	0.0037 (-0.007/0.0147)	0.4682
Heart rate (beats per minute)	65.3	66.4	1.0 (-4.6/6.7)	0.6842
Diastolic blood pressure (mmHg)	63.7	65.1	1.4 (-0.8/3.6)	0.1857
Systolic blood pressure (mmHg)	117.2	120.8	3.6 (-0.2/7.5)	0.0611

95% ci = 95% confidence interval

TABLE 5 **Population parameter estimates of the best plasma PK model (caffeinated beverage)**

Parameter (units)	Median	RSE (%)	IIV (%)
ka (hr^{-1})	180	73.3	319
ke (hr^{-1})	0.153	12.4	27
V2/F (L)	50.7	12.7	25
Q (L/hr)	240	28.1	ND
V3 (L)	12.3	11.3	ND
ALAG1 (hr)	0.23	ND	ND
CL (L/hr)	7.73	10.4	37
k_23 (hr^{-1})	4.72	35.7	25
k32 (hr^{-1})	19.6	22.8	ND
LBM COV	0.665	11.6	ND
α	0.678	6.38	ND

Parameter uncertainties and inter-individual variability (IIV) are provided when applicable.
ND, not determined; RSE, relative standard error.

TABLE 6 **Population parameter estimates of the best plasma PK model (caffeine capsule)**

Parameter	Median	RSE (%)	IIV (%)
ka (hr^{-1})	180	73.3	2549
ke (hr^{-1})	0.153	12.4	27
V2/F (L)	30.8	4.0	ND
Q (L/hr)	240	28.1	ND
V3 (L)	12.3	11.3	ND
ALAG1 (hr)	0.23	ND	ND
CL (L/hr)	4.71	10.4	27
k_23 (hr^{-1})	7.79	30.3	ND
k_32 (hr^{-1})	19.6	22.8	ND
LBM COV	0.665	11.6	ND
α	0.678	6.38	ND

Parameter uncertainties and inter-individual variability (IIV) are provided when applicable.
ND, not determined; RSE, relative standard error.

FIGURE 1 **PK model simulation of caffeine saliva concentration using data from adolescents study (dots) and previously published studies (straight continuous lines:[15,46,49,50]).** The final measurement time in the adult study is indicated by the vertical dashed line; the assay LOQ is indicated by horizontal dashed line.

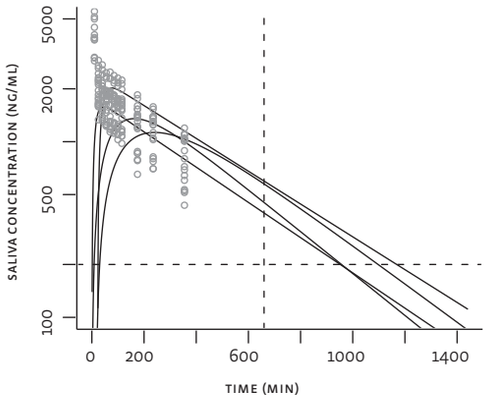

FIGURE 2 **Saccadic Peak Velocity change in least-square mean from baseline profile with 95% CI as error bars.** Rhombus: placebo; circles: caffeine.

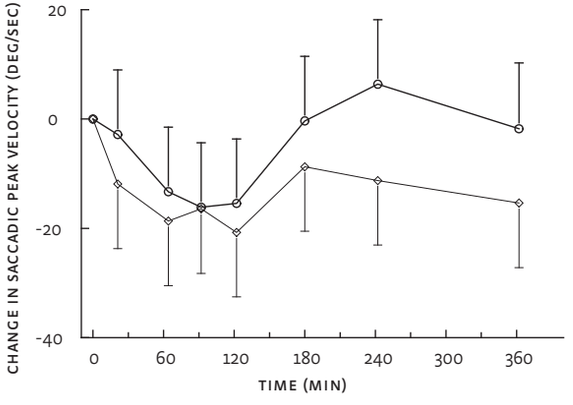

FIGURE 3 Adaptive Tracking Performance change in least-square mean from baseline profile with 95% CI as error bars. Rhombus: placebo; circles: caffeine.

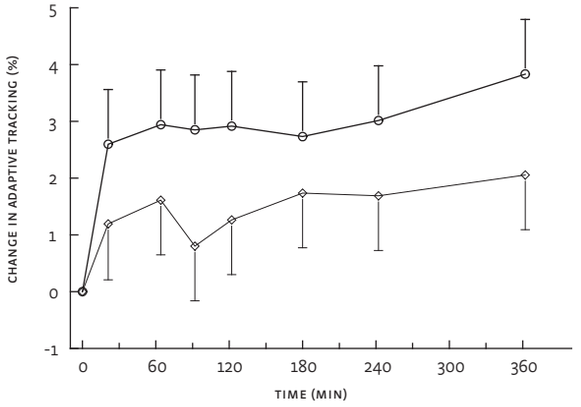

FIGURE 4 Switch Costs error rate (#3) change in least-square mean from baseline profile with 95% CI as error bars. Rhombus: placebo; circles: caffeine.

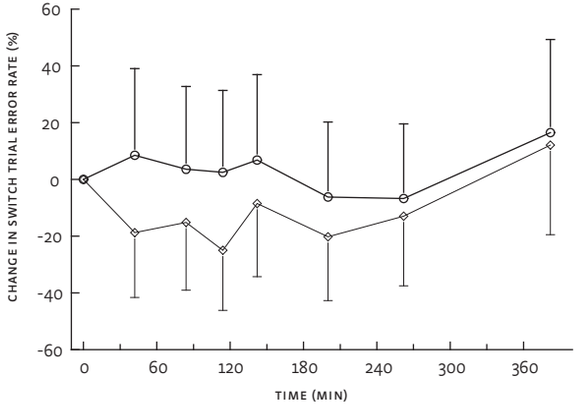

FIGURE 5 Goodness-of-fit plots for the plasma PK model. Upper left: observed (DV) versus population predicted (PRED) plasma caffeine concentrations, using fixed effects only (continuous line represents the line of unity); upper right: observed (DV) versus individual predicted (IPRED), using individual specific empirical Bayes' estimates (continuous line represents the continuous line of unity); lower left: conditional weighted residuals (CWRESI) versus individual predicted (IPRED); lower right: conditional weighted residuals (CWRESI) versus time after dose (TAD). Continuous line represents the Loess fit through the data; horizontal lines represent the mean (0) and +/- 2 standard deviations.

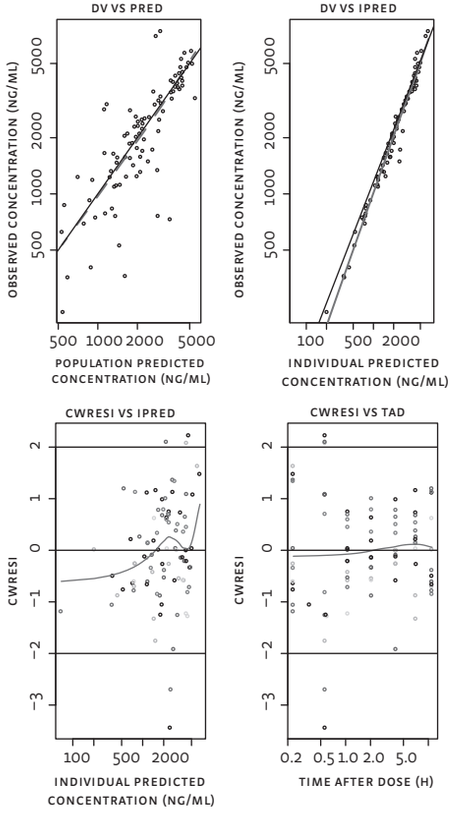

FIGURE 6 Individual caffeine plasma concentration versus time plots on a log-linear scale. Dashed lines represent the population prediction, continuous lines indicate the individual prediction, and circles represent the individual observations. S, subject number; TRT 1, treatment 1 (capsule, 200 mg caffeine); TRT 2, treatment 2 (beverage, estimated dose 90 mg caffeine)

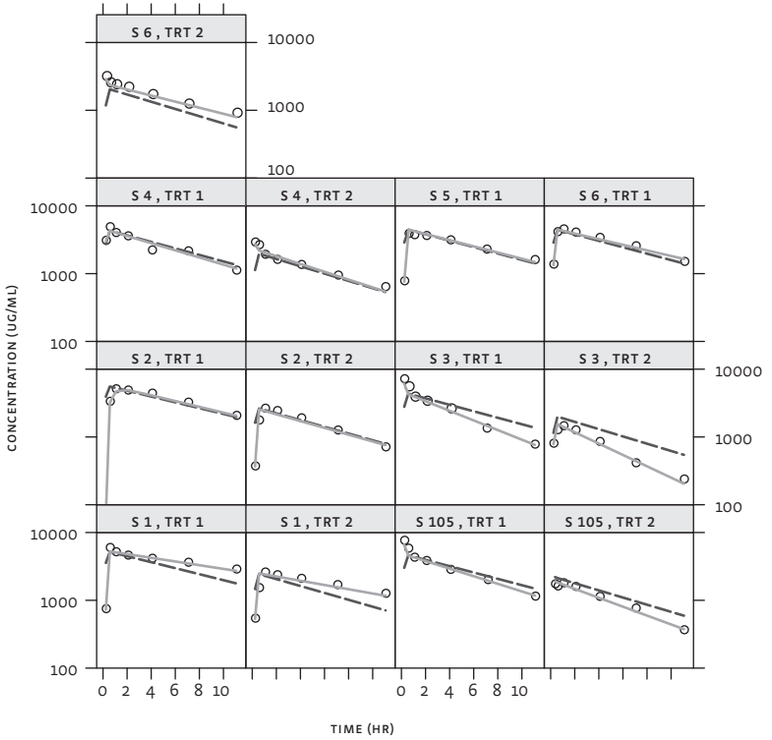

FIGURE 7 Normalized difference between caffeine plasma concentration and corresponding caffeine saliva concentration for the capsule treatment for all subjects. Saliva concentrations were divided by an estimated fraction from the relationship (0.70) in one of the sub-models. Resulting values were then subtracted from the corresponding plasma concentration, then divided by the plasma concentration. This value was then plotted against time after dose.

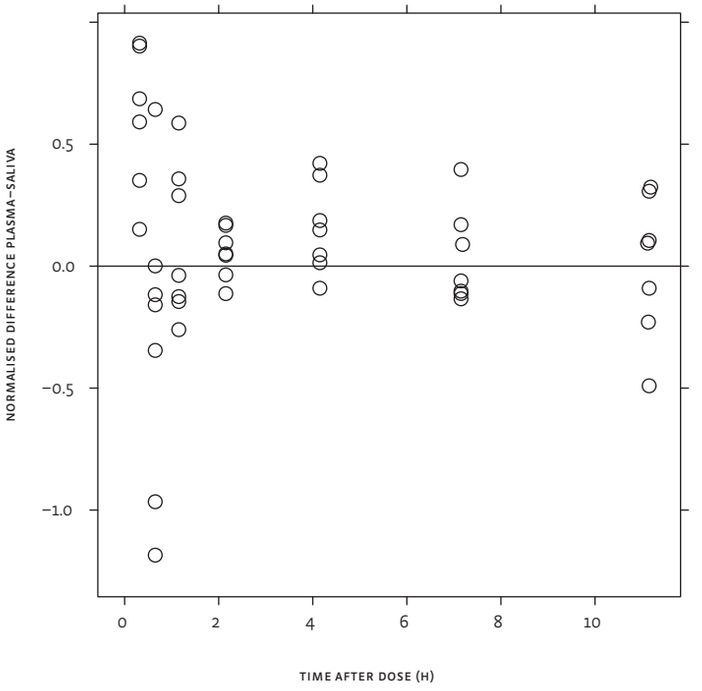

FIGURE 8 **Goodness-of-fit plots for the saliva model.** Upper left: observed (DV) versus population predicted (PRED) saliva caffeine concentrations, using fixed effects only (continuous line represents the line of unity); upper right: observed (DV) versus individual predicted (IPRED), using individual specific empirical Bayes' estimates (continuous line represents the line of unity); lower left: conditional weighted residuals (CWRESI) versus individual predicted (IPRED); lower right: conditional weighted residuals (CWRESI) versus time after dose (TAD). Continuous line represents the Loess fit though the data; horizontal lines represent the mean (0) and +/- 2 standard deviations.

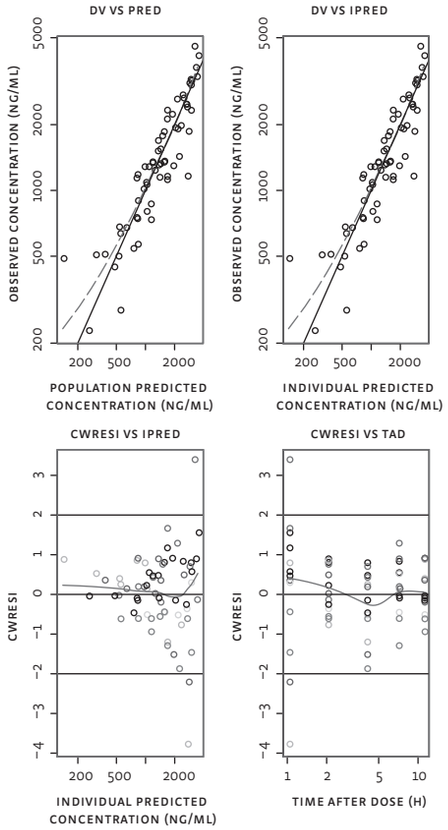

FIGURE 9 **Individual caffeine saliva concentration versus time plots on a log-linear scale.** Continuous lines represent the individual prediction and black circles the observations. Dashed line represent the plasma concentration prediction of the model and grey circles the plasma observations. S, subject number; TRT 1, treatment 1 (capsule, 200 mg caffeine); TRT 2, treatment 2 (beverage, estimated dose 90 mg caffeine)

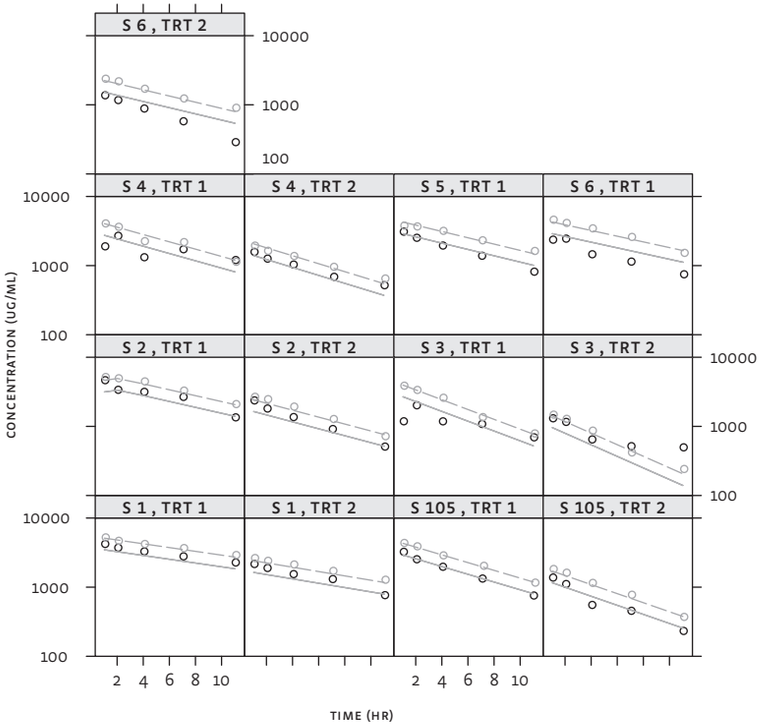

FIGURE 10 Visual predictive check for the saliva concentration after the beverage treatment in (left) adults and (right) adolescents after simulation of 1000 subjects based on the distribution of LBM in the adult and adolescent datasets. Circles represent the observations; black line represents the median prediction; dashed lines represent the 95% prediction interval. Vertical dotted line represents the 1h time point after dose; the saliva model was only based on data from this time point onwards. S, subject number; TRT 1, treatment 1 and TRT 2, treatment 2 (beverage, estimated dose 90 mg caffeine).