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PHARMACOKINETICS

Salivary caffeine concentrations are comparable to plasma concentrations in preterm infants receiving extended caffeine therapy

Correspondence Dr Nicole R. Dobson, MD, Tripler Army Medical Center 1 Jarrett White Road Honolulu, HI 96859, USA. Tel.: +1 808 433 6534; Fax: +1 808 433 6046; E-mail: nicole.r.dobson.mil@mail.mil

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Nicole R. Dobson[1,9], Xiaoxi Liu[2], Lawrence M. Rhein[3], Robert A. Darnall[4], Michael J. Corwin[5], Betty L. McEntire[6], Robert M. Ward[2], Laura P. James[7], Catherine M. T. Sherwin[2], Timothy C. Heeren[8] and Carl E. Hunt[9,10]

[1]Pediatrics, Tripler Army Medical Center, Honolulu, HI, USA, [2]Clinical Pharmacology, Pediatrics, University of Utah School of Medicine, Salt Lake City, UT, USA, [3]Newborn Medicine and Respiratory Diseases, Boston Children's Hospital, Boston, MA, USA, [4]Geisel School of Medicine at Dartmouth, Lebanon, NH, USA, [5]Slone Epidemiology Center, Boston University, Boston, MA, USA, [6]American SIDS Institute, Naples, FL, USA, [7]Pediatrics, University of Arkansas, Little Rock, AR, USA, [8]Biostatistics, Boston University School of Public Health, Boston, MA, USA, [9]Pediatrics, Uniformed Services University, Bethesda, MD, USA and [10]Pediatrics, George Washington University, Washington, DC, USA

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Keywords caffeine, pharmacokinetic model, preterm infant, saliva

AIMS

Caffeine concentrations in preterm infants are usually measured in the blood. However, salivary assays may provide a valid and practical alternative. The present study explored the validity and clinical utility of salivary caffeine concentrations as an alternative to blood concentrations and developed a novel plasma/salivary caffeine distribution model.

METHODS

Paired salivary and plasma samples were obtained in 29 infants. Salivary samples were obtained using a commercially available salivary collection system. Caffeine concentrations in the saliva and plasma were determined using high-performance liquid chromatography. A population pharmacokinetic (PK) model was developed using NONMEM 7.3.

RESULTS

The mean (± standard deviation) gestational age (GA) at birth and birth weight were 27.9 ± 2.1 weeks and 1171.6 ± 384.9 g, respectively. Paired samples were obtained at a mean postmenstrual age (PMA) of 35.5 ± 1.1 weeks. The range of plasma caffeine concentrations was 9.5–54.1 μg ml^{-1}, with a mean difference (95% confidence interval) between plasma and salivary concentrations of −0.18 μg ml^{-1} (−1.90, 1.54). Salivary and plasma caffeine concentrations were strongly correlated (Pearson's correlation coefficient = 0.87, $P < 0.001$). Caffeine PK in plasma and saliva was simultaneously described by a three-compartment recirculation model. Current body weight, birth weight, GA, PMA and postnatal age were not significantly correlated with any PK parameter.

CONCLUSIONS

Salivary sampling provides an easy, non-invasive method for measuring caffeine concentrations. Salivary concentrations correlate highly with plasma concentrations. Caffeine PK in saliva and plasma are well described by a three-compartment recirculation model.

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Non-invasive methods of monitoring drug concentrations minimize the need for blood sampling in preterm infants.
- Caffeine concentrations in preterm infants, when needed for clinical management, are measured in the blood, but salivary sampling may be a valid non-invasive alternative. Available pharmacokinetic models for caffeine have been limited to plasma concentrations.

WHAT THIS STUDY ADDS

- Salivary concentrations obtained from samples collected using a commercially available collection system correlated highly with plasma concentrations.
- Caffeine pharmacokinetics in the saliva and plasma are well described by a three-compartment recirculation model. When ascertainment of caffeine concentration is clinically indicated, salivary sampling is a simple and accurate alternative to invasive blood sampling.

Introduction

Caffeine is commonly used in the neonatal intensive care unit (NICU) for the treatment of apnoea of prematurity. It is the preferred methylxanthine for treating this condition owing to its long half-life and wide therapeutic range [1]. Measurement of caffeine concentrations in blood samples is performed when there is concern about toxicity or subtherapeutic concentrations. Salivary sampling is an attractive alternative that is non-invasive and practical, particularly in the neonatal population. Saliva is a readily available body fluid that may be repeatedly and non-invasively obtained, and this has prompted multiple studies of salivary sampling for diagnostic and therapeutic purposes in the NICU [2].

Caffeine is a neutral lipophilic molecule that diffuses readily from the blood into the saliva, independent of flow rate and salivary pH [3, 4]. Several prior studies have validated the use of saliva to monitor caffeine concentrations [5–11]. However, these studies had several limitations, including variability in the methodology used to collect and process saliva. Commercially available salivary collection systems have streamlined the process of saliva collection but these systems have not previously been utilized for the analysis of salivary caffeine concentrations in preterm infants. Moreover, salivary sampling to measure caffeine concentrations has not been performed as preterm infants approach term-equivalent age and caffeine metabolism increases.

The present study explored whether salivary caffeine concentrations were comparable to plasma concentrations when saliva was collected using a commercially available salivary collection system. The paired salivary and plasma samples were also used to develop a novel plasma/salivary caffeine recirculation model.

Methods

The present study was part of a larger clinical trial evaluating the effects of extended caffeine therapy on intermittent hypoxia in infants born preterm. This trial is registered with Clinical Trials.gov, study identifier NCT01875159. The initial study results related to intermittent hypoxia have already been published [12]. We now report the results of paired caffeine concentrations obtained in plasma and salivary samples. Six clinical centres participated in the study, and infants were enrolled from January 2013 until June 2014.

Subjects

The eligibility criteria for enrolment included: (i) preterm birth at less than 32 0/7 weeks gestational age (GA); (ii) prior history of treatment with caffeine; (iii) current age of 33–36 6/7 weeks' postmenstrual age (PMA); (iv) absence of intubation or supplemental oxygen therapy; and (v) no severe intraventricular haemorrhage (Grade 3 or 4), congenital or genetic disorder, or confirmed central nervous system infection. Institutional review board approval was obtained at each participating site. Written, informed parental consent was obtained for each infant enrolled.

Eligible subjects were enrolled once they were breathing room air and the clinical care team anticipated discontinuing routine caffeine therapy (maintenance dose of 5–10 mg kg^{-1} day^{-1}) within the next week. The day after the clinical care team discontinued routine caffeine, enrolled infants were started on study caffeine orally at a dose of 10 mg kg^{-1} day^{-1}. This dose was further increased at 36 weeks' PMA to compensate for the increased rate of caffeine metabolism that occurs with maturation after 36 weeks [13]. At 36 weeks' PMA, infants were randomly assigned to receive either 14 mg kg^{-1} day^{-1} or 20 mg kg^{-1} day^{-1} given b.i.d.

Plasma and salivary caffeine samples

All plasma and salivary samples represented trough samples, obtained <1 h prior to the next caffeine dose. In addition to the serial salivary caffeine concentrations obtained as part of the overall protocol, we obtained one paired plasma and salivary caffeine sample from each subject to assess the correlation between these concentrations. To compare salivary and plasma concentrations over a wide range of caffeine concentrations, the paired samples were obtained at varying PMAs and caffeine doses. In some cases, the paired samples were obtained prior to randomization and reflect the caffeine dose that the subject was receiving as part of routine clinical practice. Whenever possible, the paired samples were obtained concurrently with a clinically indicated blood sample. For the plasma sample, 0.6 ml of blood was collected in an anticoagulant tube and centrifuged, and the plasma (average volume 0.3 ml) was frozen at −20°C pending analysis.

Salivary samples were collected using the Salimetrics Salivary Collection System with children's swabs (Salimetrics, LLC, State College, PA, USA). The swab was placed in the infant's mouth for 60–90 s until the lower third of the swab was saturated with saliva; the saturated end of the swab was then placed in the collection tube insert and cut off with

clean, sharp scissors. The process was repeated approximately 2 min later with the other end of the swab, which was placed into the same collection tube insert. The second swab was utilized to ensure the collection of an adequate volume of saliva. The tube was then capped tightly and centrifuged for 15 min at 1800 *g* so that the liquid from the saliva drained through a small hole in the bottom of the top insert tube into the lower collection tube. After centrifugation, the upper insert tube was removed from the collection tube and the saliva was frozen at −20°C until analysis. A volume of 0.2–0.5 ml of salivary liquid was collected using this technique.

Measurement of caffeine concentrations

All frozen plasma and salivary samples were shipped overnight to the Pediatric Pharmacology Laboratory (Dr Laura James) at Arkansas Children's Hospital for batch processing. Caffeine concentrations in salivary and plasma samples were analysed using high-performance liquid chromatography based on a modification of a previously published validated method [14]. Briefly, the protein in the saliva and plasma samples was precipitated with ethyl acetate. The supernatant was removed and evaporated under nitrogen, reconstituted in a mobile phase and filtered through a 0.2 micron filter before injection. Chromatography was performed using a Waters (Milford, MA, USA) Alliance 2695 Separations Module equipped with a Waters 2996 Photodiode Array detector set at 280 nm and a Waters Symmetry C18 250 mm column. The mobile phase consisted of acetonitrile : acetic acid : water at a ratio of 100:1:899. The column temperature was set to 37°C and the flow rate was 1.2 ml min^{-1}. Standard curves were linear ($R > 0.99$) for the range of 6.25–200 μg ml^{-1}. Controls in the serum and saliva were run at concentrations of 6.25 μg ml^{-1}, 12.5 μg ml^{-1} and 50 μg ml^{-1}, with coefficients of variation from 5.1% to 11.5%.

Statistical analyses

Demographic data are presented as means ± standard deviations unless otherwise noted. The agreement between plasma and salivary concentrations is presented as a scatterplot and Pearson's correlation coefficient was calculated. Agreement is also described using a Bland–Altman plot, which plots the difference in plasma and salivary concentrations by the average of the two concentrations, and 95% limits of agreement. The Bland–Altman plot can be used to examine bias [does the 95% confidence interval (CI) of the mean differences include 0], to assess precision (the 95% limits of agreement) or whether agreement changes across the range of caffeine concentrations (if there is a trend in the plot). The population pharmacokinetic (PK) model was developed using NONMEM 7.3 (ICON plc; Dublin, Ireland) and PsN 4.4.8 (La Jolla, CA, USA). The ADVAN6 subroutine was used to build the customized mathematical model. The FOCEI method was applied to obtain the final estimates.

Results

The study population included 29 infants with a gestational age of 27.9 ± 2.1 weeks, a birth weight of 1171.6 ± 384.9 g and a postnatal age of 35.5 ± 1.1 weeks. Caffeine doses (mg kg^{-1} day^{-1}) at the time of the paired plasma and salivary samples are summarized in Table 1. At the time of paired sampling, infants were receiving a range of caffeine doses depending on PMA and randomization group. Blood and salivary samples were obtained simultaneously in 21 infants. The interval between blood and salivary samples was ≤15 min in 27 of 29 infants, and 30–45 min in the other two infants. The median (interquartile range) body weight at the time of paired sampling was 2100 g (1600, 2567). Caffeine therapy was well tolerated and no infants were exhibiting signs of toxicity.

Plasma caffeine concentrations ranged from 9.5–54.1 μg ml^{-1}. There was good agreement between salivary caffeine concentrations and plasma concentrations over the entire range of values, with a Pearson's correlation coefficient of 0.87 (Figure 1; $P < 0.001$). The mean of the differences

Table 1

Caffeine dosing at time of paired blood and salivary samples

Caffeine dose (mg kg^{-1} day^{-1})*	Number of subjects
5	1
10	21
14	5
20	2

*Enrolled infants were started on study caffeine at a dose of 10 mg kg^{-1} day^{-1} when the clinical team discontinued routine caffeine. One infant had paired sampling performed prior to being started on study dosing. At 36 weeks' postmenstrual age, infants were randomized to receive either 14 mg kg^{-1} day^{-1} or 20 mg kg^{-1} day^{-1} divided twice a day.

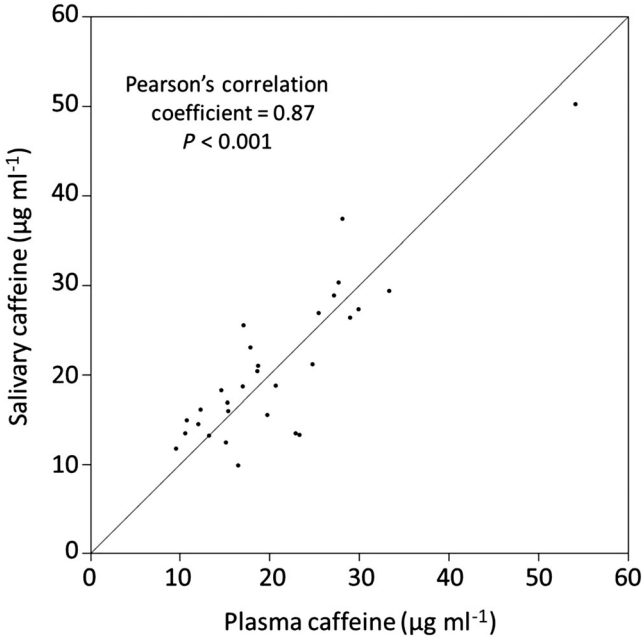

Figure 1

Scatterplot of pairs of salivary and plasma caffeine concentrations collected in 29 preterm infants. The line of identity is drawn through equal concentrations of caffeine in salivary (y-axis) and plasma (x-axis) samples. Salivary caffeine concentrations correlated strongly with plasma concentrations over a wide range of caffeine concentrations

(95% CI) between plasma and salivary concentrations was −0.18 (−1.90, 1.54), showing no significant bias between the two measures (Figure 2). The 95% limits of agreement (i.e. precision) were −9.36, 9.00, and the agreement appeared to be consistent across caffeine concentrations.

PK model

The caffeine PK in plasma and saliva is simultaneously described by a three-compartment recirculation model. The model consists of a central plasma compartment, a salivary depot compartment and an absorption depot compartment (Figure 3). The model is parameterized as plasma volume of distribution (V1), salivary depot volume of distribution (V2), plasma clearance (CL1), plasma/salivary redistribution clearance (CL2), salivary secretion rate (CL3) and absorption rate constant (KA). Between-subject variability was initially examined on each PK parameter and was retained on CL1 and V1 in the final model. Additive, proportional and combined residual error structures were examined in the model. Additive error structure improved the model fitting the most and was applied in the final model. Current body weight, birth weight, gestational age, PMA and postnatal age were screened for potential covariates, and were not detected with statistical significance. The final parameter estimates are listed in Table 2. The salivary secretion rate (CL3) and absorption rate constant (KA) were fixed to 0.006 l h^{-1} and 1.48 h^{-1}, respectively, based on literature data [13, 15]. Complete absorption was assumed [13]. All PK parameters were estimated with high confidence. Goodness-of-fit plots were generated for observed plasma and salivary concentrations *vs.* population-predicted and individual predicted values (Figure 4A, B), and weighted residuals *vs.* population-predicted values and time after first dose (Figure 4C, D; Figures S1, S2). Visual predictive check analysis (sample number = 1000) was also conducted and returned 93.1% coverage within the 90% predictive interval (Figure S3).

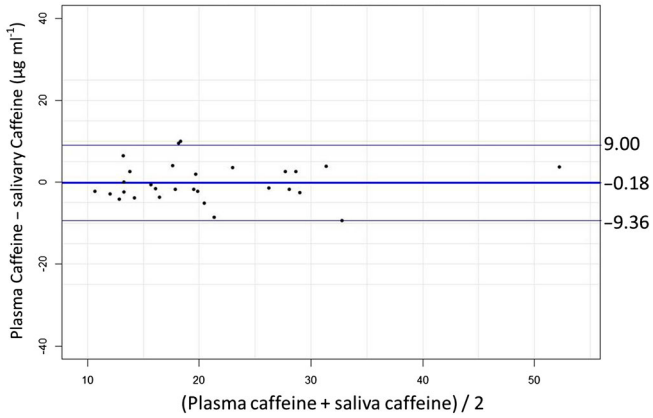

Figure 2

Bland–Altman plot depicting the mean of the differences and 95% limits of agreement between salivary and plasma concentrations. The bold line represents the mean of the differences (bias). The upper and lower lines represent the limits of agreement (precision)

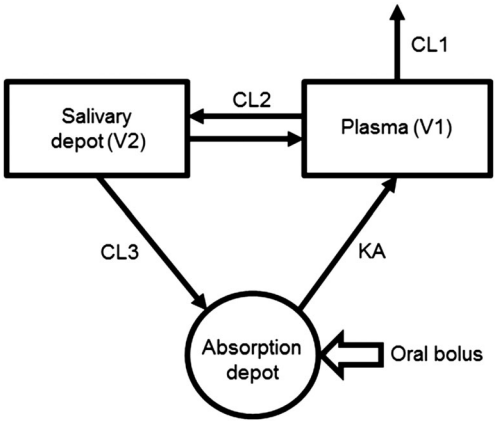

Figure 3

Pharmacokinetic model structure. CL1, plasma clearance; CL2, plasma/salivary redistribution clearance; CL3, salivary secretion rate; KA, absorption rate constant; V1, plasma volume of distribution; V2, salivary depot volume of distribution

Discussion

Our primary finding was that salivary caffeine concentrations were highly correlated with plasma concentrations over a wide range of caffeine concentrations in preterm infants approaching term-equivalent age. In addition, the distribution of caffeine between plasma and saliva was well described by a recirculation PK model. This model explains the caffeine concentration correlation between plasma and saliva, and can potentially be utilized to predict plasma caffeine concentrations from salivary measurements in future investigations.

Table 2

Summary of final pharmacokinetic model parameter estimates

Pharmacokinetic parameters	Estimates	95% CI
Plasma volume of distribution (V1, l)	0.966	0.509–1.423
Salivary depot volume of distribution (V2, l)	0.00954	0.004–0.015
Plasma clearance (CL1, l h^{-1})	0.0167	0.014–0.019
Redistribution clearance (CL2, l h^{-1})	0.00588	0.005–0.006
Salivary secretion rate (CL3, l h^{-1})	0.006	–
Absorption rate constant (KA, h^{-1})	1.48	–
Between-subject variability		RSE%
ω^2_{CL1}	0.0729	52.80%
ω^2_{V1}	0.823	25.40%
Residual error (μg ml^{-1})	9.47	26.10%

CI, confidence interval, RSE%, relative standard error, ω^2_{CL1}, variance of CL1 between-subject variability; ω^2_{V1},variance of V1 between-subject variability

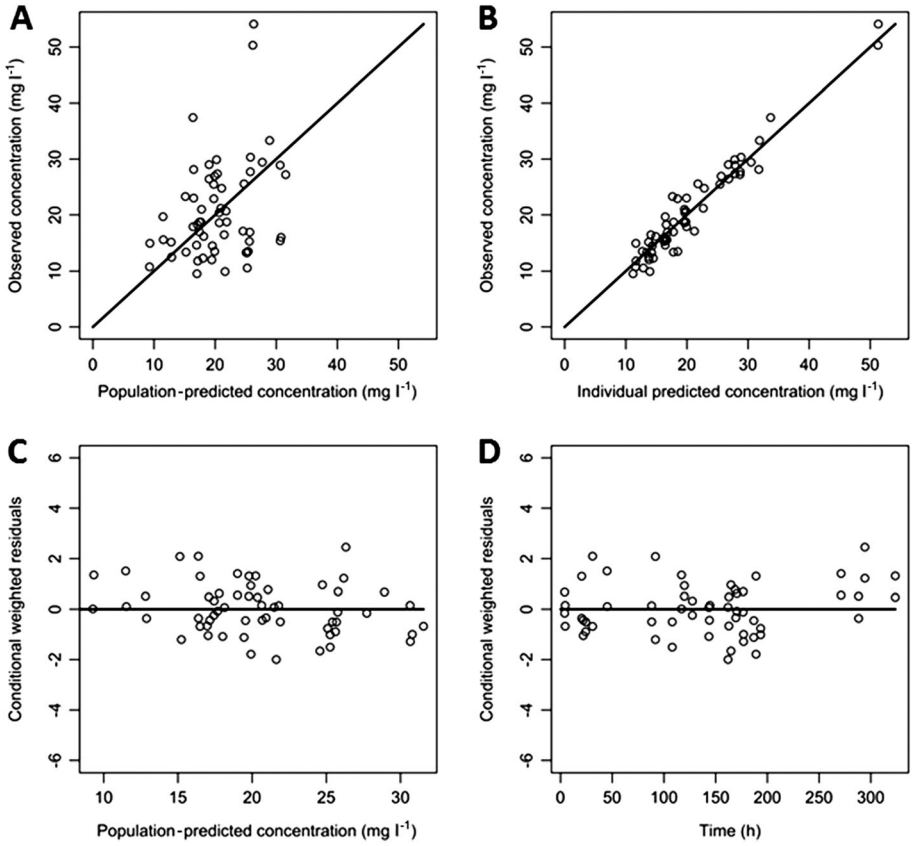

Figure 4

Goodness-of-fit plots. Diagnostics for both plasma and salivary concentratons are presented. (A) Observed concentration *vs*. predicted population concentration. (B) Observed concentration *vs*. predicted individual concentration. (C) Conditional weighted residuals *vs*. predicted population concentration. (D) Conditional weighted residuals *vs*. time after first dose

As caffeine concentrations are typically measured only when clinicians are concerned about subtherapeutic or toxic concentrations, our observed 95% limits of agreement between plasma and saliva (−9.36, 9.00) were more than sufficient for clinical purposes.

Caffeine is metabolized in the liver by four cytochrome P450 isoforms. The N7-demethylation process is the primary caffeine metabolic pathway in premature infants and the enzymes mature progressively with increasing gestational age at birth and increasing PMA [16]. Caffeine clearance increases with increasing weight and postnatal age [17, 18]. In current clinical practice for infants less than 32 weeks' PMA, routine caffeine maintenance doses of 5–10 mg kg^{-1} day^{-1} are typically sufficient to achieve blood concentrations of 8–20 μg ml^{-1} and infants have been shown safely to tolerate caffeine concentrations as high as 50–84 μg ml^{-1} [16, 19]. Because of the wide therapeutic index with a low potential for toxicity, blood caffeine concentrations are rarely monitored in current practice except when concerned that the concentration is subtherapeutic or excessive [20]. However, routine caffeine concentration monitoring will be especially important in preterm infants after 32 weeks, and especially after 35 weeks, PMA as concentrations progressively decrease owing to increasing caffeine metabolism, and there is limited information about the caffeine doses that are needed at this PMA to achieve or maintain effective concentrations [12, 13]. More detailed information about caffeine metabolism as preterm infants approach term-equivalent age is now relevant because there is an increasing tendency to continue caffeine treatment beyond 34–35 weeks' PMA, in part owing to recent data related to intermittent hypoxia [12]. Intermittent hypoxia in extremely preterm infants in the first 2–3 months after birth is associated with a higher risk of death or neurodevelopmental disability at 18 months, and additional studies are needed to determine if persisting intermittent hypoxia after stopping routine caffeine treatment is associated with further morbidity risk [21]. As invasive blood sampling is often not clinically indicated at these older PMAs, the ability to measure caffeine concentrations non-invasively in the saliva will provide a simple and painless alternative both for clinicians and researchers.

In regard to the routine use of caffeine in the early postnatal weeks prior to 33–34 weeks' PMA, a recent retrospective study in infants <30 weeks' gestation associated higher average caffeine concentrations with a decreased duration of ventilation, lower chronic lung disease rates, a shorter duration of supplemental oxygen use and a shorter length of stay [22]. Clinicians using higher caffeine doses will be more inclined to employ caffeine concentration monitoring in routine clinical practice in the NICU to address concerns of potentially

excessive levels. The results of the present study support the use of salivary sampling for the therapeutic monitoring of caffeine concentrations as infants are approaching term-equivalent age.

Non-invasive methods of monitoring drug concentrations are appealing because they minimize the amount and frequency of invasive blood sampling in preterm infants. Salivary sampling provides an easy, non-invasive alternative to blood sampling. Neonates have higher salivary flow rates than adults [23], and saliva can be collected without causing discomfort to the infant. Prior studies have investigated the use of saliva to measure caffeine concentrations in preterm infants [5–11]. Early studies were limited by small numbers of patients and a narrow range of caffeine doses, ranging from 3–5 mg kg^{-1}, with one group suggesting that salivary concentrations were only useful if caffeine concentrations were lower than 8 μg ml^{-1} [8]. More recent studies investigated a wider range of caffeine doses. Lee and colleagues enrolled infants born at <32 weeks' gestation in the first week of life [10]. The infants were randomized to one of three caffeine doses (3, 15 or 30 mg kg^{-1} administered intravenously), leading to a wide range of caffeine concentrations. Unstimulated mixed saliva was collected by vacuum aspiration from the floor of the mouth into a plastic silicon-coated tube [10]. Researchers collected 131 paired serum and salivary samples for analysis of caffeine content by high-performance liquid chromatography. The mean ratio of saliva-to-serum concentrations was 0.924, with no significant difference in precision between the data samples and a small negative bias for saliva *vs*. serum monitoring [10]. While our study findings were similar, the method used in the present study was less labour intensive. In addition, the use of a commercially available salivary collection system allowed collection of adequate volumes of saliva in approximately 5 min, compared with to up to 30 min for the previous study [10], and thus minimized risk of infant discomfort.

Another study comparing salivary and plasma caffeine concentrations employed three different techniques to collect saliva to determine the best collection method [6]. They compared unstimulated saliva collection to two different techniques of dilute citric acid stimulation (dilute citric acid placed on gauze at the time of collection *vs*. dilute citric acid placed directly in the cheek pouch 5–10 min prior to saliva collection). For all three methods, saliva was collected using a 2 cm by 4 cm nonwoven gauze attached to a cotton swab that was placed in the cheek for 5–15 min. Saliva was then expressed using a 2 ml syringe into a collection tube. The investigators collected 237 paired plasma/saliva pairs from 140 infants ranging in gestational age from 24 weeks to 34 weeks. The mean postnatal age was 27 days, and the doses prescribed ranged from 1.3 mg kg^{-1} day^{-1} to 5.1 mg kg^{-1} day^{-1}. For all collection methods, there was good correlation between salivary and plasma concentrations but the saliva sampling method using citric acid in the infant's cheek pouch before collection showed the strongest correlation with plasma caffeine concentrations [6]. However, this technique carries the risk of dilution of the sample by citric acid solution, which may reduce repeatability [24]. In the present study using a commercially available salivary collection system, adequate volumes of saliva were collected without the administration of citric acid.

The Salimetrics salivary collection system was a convenient method for saliva collection, with adequate volumes of saliva obtained for analysis from all samples collected. Salivary sampling is suitable for repetitive sampling, especially when serial sampling is needed and in the context of research studies, when invasive blood sampling may not be acceptable or practical. The PK model allows estimation of plasma concentrations from saliva samples, creating the potential for easy, non-invasive caffeine concentration monitoring in the NICU.

Other minimally invasive techniques have been used to determine caffeine concentrations in preterm infants. A recent study determined caffeine concentrations from dried blood spots [25]. This technique reduces the required blood sample volume but still requires a painful procedure for sample collection. Another study analysed these concentrations in urine and found good correlation with plasma concentrations [26].

Other PK models for caffeine have previously been published [13, 17, 25, 27]. However, only plasma caffeine concentrations were measured in these studies. All models assumed a one-compartment structure that explained the caffeine PK in plasma. A significant improvement in our model is that the salivary caffeine measurements were also incorporated. The salivary caffeine PK and its association with plasma caffeine PK are therefore described simultaneously. In previous publications, body weight and/or postnatal age were included as covariates for clearance and volume of distribution. In some cases, such incorporation was based on the improvement of the model fitting, which was highly data dependent. In other cases, the weight and postnatal age impact were simply assumed. In the present study, weight and postnatal age were initially examined as potential covariates but were not included in the final model because they resulted in an insignificant improvement in model fitting. Nonetheless, the plasma PK parameters were estimated at concentrations that were comparable with those in previous studies (Table 3).

Caffeine is secreted into breast milk and may produce measurable serum concentrations in neonates [28–31]. Saliva collected soon after a breastfeeding session may overestimate the caffeine concentration because of the presence of caffeine in the breast milk after maternal caffeine ingestion. All of our salivary samples were collected at least 2 h after the last enteral feeding, to minimize any potential for contamination from breast milk, and there was a strong correlation with plasma concentrations. Additional limitations of the present

Table 3

Comparison of published pharmacokinetic parameter estimates

	Clearance (l h^{-1})	Volume of distribution (l)
Lee *et al.* [17]	0.012	2.2
Patel *et al.* [25]	0.015	1.29
Charles *et al.* [13]	0.016	1.76
Thomson *et al.* [27]	0.015	0.82

The same mean body weight (2099 g) and postnatal age (27.9 days) were assumed when calculating the values.

study include a relatively small number of samples and a limited number of caffeine concentrations exceeding 30 μg ml^{-1}, although the correlation between plasma and salivary concentrations was the same for these as at lower concentrations.

In conclusion, the present study showed that salivary caffeine concentrations correlate highly with plasma concentrations over a wide range of caffeine concentrations in preterm infants approaching term-equivalent age. Future studies will further validate the three-compartment recirculation PK model as a useful tool to predict plasma caffeine concentrations using salivary concentrations. Our results confirm that salivary samples are an appropriate alternative to blood for measuring caffeine concentrations when clinically indicated, including when serial sampling is needed or blood sampling is not practical or feasible.

Competing Interests

The views expressed in this manuscript are those of the authors and do not reflect the official policy or position of the Department of the Army, Department of Defense or the US Government. All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: CEH and BLM had support from the American SIDS Institute for the submitted work; LMR reports grants from PCORI and non-financial support from Masimo outside the submitted work; LPJ is part owner of Acetaminophen Toxicity Diagnostics, LLC, which is funded by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) STTR/2R42DK079387; LPJ receives salary support for research from the National Center for Advancing Translational Sciences (NCATS) awarded to the Translational Research Institute at the University of Arkansas for Medical Sciences (UL1RR029884). All other authors have no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years nor other relationships or activities that could appear to have influenced the submitted work.

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Contributors

NRD, LMR, RAD, MJC, BLM, RMW and CEH designed the study. NRD, LMR, RAD, BLM and CEH recruited subjects and conducted the clinical project. XL, CMTS, RMW and LPJ conducted the pharmacokinetic analysis. LPJ contributed essential reagents and assayed caffeine concentrations. XL, CMTS, TCH and MJC analysed the data. NRD, XL, MJC, BLM, CMTS and CEH drafted the manuscript. All authors reviewed the manuscript drafts and approved the final version for submission.

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Supporting Information

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Figure S1 Goodness-of-fit plots. Diagnostics for plasma concentratons are presented. (A) Observed concentration *vs*. predicted population concentration. (B) Observed concentration *vs*. predicted individual concentration. (C) conditional weighted residuals *vs*. predicted population concentration. (D) Conditional weighted residuals *vs*. time after first dose
Figure S2 Goodness-of-fit plots. Diagnostics for salivary concentratons are presented. (A) Observed concentration *vs*. predicted population concentration. (B) Observed concentration *vs*. predicted individual concentration. (C) Conditional weighted residuals *vs*. predicted population concentration. (D) Conditional weighted residuals *vs*. time after first dose
Figure S3 Visual predictive check plot. Solid line, 50th percentile of simulation; dashed lines, 90% predictive interval