

# The Usefulness of Saliva in Therapeutic Drug Monitoring of Caffeine in Preterm Infants.

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## Research Article

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## Abstract

### Aim

To verify if the concentrations of caffeine in saliva are comparable to the serum concentrations in preterm infants treated with caffeine for apnoea of prematurity.

### Methods

Prospective observational study. Eligible patients were newborn infants < 37 weeks of gestational age treated with oral or intravenous caffeine for apnoea of prematurity. Two paired samples of saliva-blood were collected per patient. Tube solid phase microextraction coupled on-line to capillary liquid chromatography with diode array detection were used for analysis.

### Results

A total of 47 newborns with a median gestational age 28 [26–30] weeks and mean of  $1.11 \pm 0.4$  kg of birth weight. Median postmenstrual age, when samples were collected, was 31 [29–33] weeks. Serum caffeine median levels of  $19.30 \mu\text{g/mL}$  [1.9–53.90] and salivary caffeine median levels of  $16.36 \mu\text{g/mL}$  [2.20–56.90] were obtained. There was a strong positive Pearson's correlation between the two variables  $r = 0.83$  ( $p < 0.001$ ).

### Conclusion

The measurement of caffeine salivary concentrations after intravenous or oral administration offers an alternative to serum caffeine monitoring in apnoea of prematurity.

### Introduction

Apnoea of prematurity (AOP) is one of the most frequently conditions in neonatology, affecting nearly all of preterm infants with a birth weight < 1000 g [1]. Its incidence is inversely related to the gestational age (GA) [1, 2].

Since 1970's, methylxanthines have been employed as therapies for AOP [3], being caffeine the drug of choice to treat AOP [3] because it has a fairly wide therapeutic window, better intestinal absorption and a longer half-life.

Caffeine is a lipophilic molecule that is rapidly absorbed and distributed throughout the body and excreted in urine and saliva. Clinically, caffeine is administered orally and intravenously for the treatment of AOP. In neonates, caffeine causes a peak in concentrations 0.5-2 h after oral administration and the plasma half-life ranges between 65–100 h. Of note, there is a remarkable shortening of its half-life with neonatal maturation. The effective therapeutic concentrations in plasma are 13–25 mg/L; however, occasionally levels of 26 to 40 mg/L may be required. Generally, no safety problems have been described with plasma values below 50 mg/L [4].

Despite caffeine is considered the most efficacious drug for the treatment of AOP [5], there is no consensus in its optimal loading and maintenance dose among different neonatal units and some questions about outcomes and safety of its dosage are still unresolved [6–8]. Therefore, precise monitoring of caffeine levels may be essential to characterize its pharmacokinetic behaviour in unresponsive patients or when we use higher doses.

Analytical methods are currently available for the determination of caffeine and its metabolites in biological fluids, including saliva; however, plasma levels are the most commonly employed [9]. But blood collection in neonates has several limitations such as the amount of blood available for analysis and repeated painful procedures. The availability of easily accessible non-invasively obtained biofluids with caffeine levels that would correlated with the plasmatic would be a considerable step forward to achieve better therapeutic results avoiding unwanted side-effects [10]. It will also improve our knowledge about the pharmacokinetics of the most used medication in neonatal units.

We aimed to assess if there is a positive correlation between serum and saliva concentrations of caffeine in preterm infants treated for AOP.

### Population And Methods

#### *Participants and treatment*

All newborn infants <37 weeks GA admitted to the NICU of the University and Polytechnic Hospital La Fe, Valencia (Spain) treated with caffeine citrate for AOP between December 2017 and August 2018 were eligible. The study protocol was approved by the Institutional Review Board of our

hospital (code: GIP-CAF-2017-01/408). Written informed consent was obtained from the parents before inclusion. Patients with major malformations or chromosomopathies or whose parents refused to participate were excluded.

Patients included in this study received, according to the AOP protocol of our NICU, a loading dose of 20 mg/kg and a maintenance dose of 7-10 mg/kg/24h of caffeine citrate (Peyona®, Chiesi Farmaceutici; Parma, Italia) based on clinical needs. Adverse reactions possibly related to caffeine treatment were recorded.

### ***Samples collection and storage***

Two paired samples of saliva and blood were collected per patient. The first paired samples were obtained 72 h after first caffeine dose and the second, almost 7 days thereafter. Salivary and blood samples were obtained <1 h prior to the next caffeine dose coincident with clinical blood sampling and not exceeding 10 minutes between samples. The second pair of samples was not withdrawn if there was not clinical indication of blood test and caffeine had been suspended for >7 days.

Saliva samples (10 µL) were collected using cellulose spears (EYETEC®, North Yorkshire, UK) placed in the mouth for 1-3 minutes until saturation, then frozen at -20°C. Just before analysis, spears were centrifuged 3 minutes at 4000G, and the supernatant was collected. Salivary secretion stimulators were not used.

Blood samples (0.1-0.15 mL) were collected in dry tube (Mini-collect®, Greiner Bio-One, Kremsmünster, Austria) and centrifuged at 1500G, 10 minutes, not refrigerated, immediately after being collected, then 50-100 µL were aliquoted into 1.5 mL Eppendorf and frozen at -20°C until analyses.

### ***Analytical method***

MINTOTA Research Group Laboratory analyzed caffeine samples according to a previously published and validated method by Ponce-Rodriguez et al [11].

### ***Statistical analysis***

To determine differences a logistic regression analysis was performed. Three Pearson's correlations were calculated between serum and saliva (all patients, IV caffeine and O caffeine).

To assess agreement between salivary and plasma concentrations, three Bland-Altman plots were drawn: global sample, patients with IV caffeine and O caffeine. Additionally, multivariable linear regression models were performed including sex, birth weight, GA, postmenstrual age (PMA) and caffeine dose. Due to multiple observations of serum and saliva in the same patient, the linear regressions were extended with the individuals as random intercept to correct for the nonindependence. Other drugs administered were included in the model as a sensitivity analysis. R software (v.3.5.1) and packages, *clickR* (v.0.4.22), *lme4* (v.1.1-18-1) and *blandr* (v.0.5.1) were used for the descriptive, inferential analyses, and Bland Altman.  $P < 0.05$  was considered to indicate statistical significance (2-tailed test).

## **Results**

We included 47 newborns with a median GA of 28 [26 -30] weeks and a mean of  $1.11 \pm 0.4$  kg of birth weight. Patients had a median PMA of 31 [29 - 33] at the moment of sample collection. Sociodemographic and clinical characteristics are shown in Table 1. Two pairs of saliva-blood samples were withdrawn from 29 newborns and one pair in 18. Mean caffeine dose at the time of collection was  $8.49 \pm 2.22$  mg/kg/day.

Serum caffeine median levels of 19.30 (range: 1.9 – 53.9 µg/mL) and salivary caffeine median levels of 16.36 (range: 2.20 – 56.90 µg/mL) were obtained. There was a strong positive Pearson's correlation between the two variables  $r=0.83$ ,  $p<0,001$  (Figure 1A). Furthermore, we compared the correlation between patients who received intravenous or oral caffeine. Hence, 42.6% received IV caffeine ( $r= 0,727$ ,  $p<0.001$ ) and 57,4% oral caffeine ( $r=0.904$ ,  $p<0.001$ ) (Figure 1B and 1C, respectively). Mean of serum and salivary levels according VO/IV administration were also calculated (Table 2).

Through a multivariate predictive model including all the patients, and saliva caffeine levels (estimate= -1.838 IC95% [-3.053; -0.623],  $p=0.003$ ). As a measure of model performance and bias we obtained a = 0.70 and root-mean squared error RMSE = 4.96. Referring to the IV model, we found a moderate variance explained, = 0.489, RMSE = 5.711. O model showed the best adjust with  $R^2 = 0.833$ , RMSE = 3.843. The regression analysis including all patients yielded a model expressed by an equation:

$$E(\text{serum}) = 38.657 + 0.656 * \text{saliva caff level} - 3.41 * \text{birth weight} - 0.054 * \text{GA} - 0.972 * \text{PMA} \\ - 0.717[\text{VO}] + 4.692 * \text{actual weight} + 0.159[\text{Male}]$$

The differences between route of caffeine administration were individually analysed using the Bland–Altman test. Taken all patients into consideration, Figure 2A shows that the mean difference was -2.92 with a confidence interval for limits of agreements of [9.24; -15.09]. The same procedure was carried out for IV and VO. Intravenous administration showed a mean difference of -2.51 but the largest limits of agreements [12.76; -17.81] (Figure 2B). Oral administration presented a mean difference of -3.21 with the lowest limits of agreements [6.28; -12.70] (Figure 2C). So, O administration showed better agreements because although the difference from the mean is slightly greater, the limits of agreement are much smaller. We found a trend in the three analyses to increase the differences as the mean increases.

In addition, when PMA was compared in O versus IV administration there were significant differences ( $p=0.044$ ), (Figure 3A) but no for weight at sample collection ( $p=0.327$ ) (Figure 3B). Any adverse events were reported.

## Discussion

In neonates, AOP cast serious clinical concerns about the negative effects of dysoxia [1], secondary oxidative stress damage with lipid peroxidation and subsequent neurological problems [12]. Caffeine for AOP treatment is the most widely used drug in neonatology. Its use is associated to reduction of bronchopulmonary dysplasia [13, 14], and improvement of long-term neurodevelopmental outcome [15–17].

The wide caffeine safety interval restricts the caffeine level determinations, performing them just when there is a suspicion of intoxication or a poor therapeutic response with an adequate dosing. Nevertheless, routinely neonatologists adjust caffeine dose in AOP according to the expected response raising it even above the recommended doses to avoid mechanical ventilation, or lowering it when a suspect side effects appear [8].

Our study shows a high correlation between saliva and serum caffeine concentrations in premature infants. Pharmacokinetics and pharmacodynamic studies are necessary in neonates where available data are scarce in most of the drugs used. Because neonatology covers a short period of life, but it implies significant physiological changes. Supporting this assumption, we found a negative association between PMA and serum/saliva caffeine levels. However, the recommended caffeine dosage does not vary with maturity as in other drugs in neonatology; only some pharmaceutical guide changes the dosage schedule in the more mature infants. Even in studies with mathematical pharmacokinetic simulation models, they suggest increasing the dose of caffeine at a rate of 1 mg / kg each week of life to maintain the levels in range, despite not being carried out in routine clinical practice [18, 19]. We should also take into account the pharmacogenetics already described and the unknown information [20] about relevant polymorphisms for this drug related to clinical effects in the individualized prescription in neonates with immature metabolism of drugs, despite its renal elimination.

The option to measure non-invasively caffeine concentration by saliva sampling, will provide a free-blood and painless alternative to blood samples. Ours observed 95% limits of agreement between plasma and saliva regardless of the route of administration (-15.09; 9.20) are suitable for clinical purposes, being able to extend the indication of requesting caffeine levels. We found better correlation in O administration and at lower levels of serum/saliva caffeine concentration, as other studies [21–24].

This better correlation with oral administration can be explain by a more stable plasma levels when using this route given the excellent bioavailability of oral caffeine. But it also may be related to more mature patients receiving the oral treatment (significant differences in Fig. 3). Really, older patients eliminate caffeine faster, obtaining lower levels, and so, better correlation. It is also possible that they were more stable and probably received fewer concomitant drugs. We also have little data on pharmacological interferences in neonatology. We rule out interferences with other administered drugs using a linear regression/predictive model. In clinical practice, this better correlation reinforces the recommendation for oral caffeine use, which minimizes infectious risk and it is not related to alterations in cerebral perfusion such as rapid intravenous bolus [25].

Several studies show also a highly correlation of salivary drug concentration with serum drug concentration of caffeine in neonates (Table 2), but not in such a premature population and in the daily practice. Khanna et al. [21] reported a small study in 7 premature newborns of paired caffeine samples of plasma and saliva after oral administration. Significant correlation in caffeine concentrations determined by high pressure liquid chromatography (HPLC) were observed but only for serum levels less than 8  $\mu\text{g}/\text{mL}$  which is at the bottom of recommended range therapeutic for caffeine. Moreover, samples not represented by trough samples because they were obtained four to six hours after administration.

Lee et al.[22] enrolled 59 preterm infants < 32 weeks GA in the first 7 days of life and randomized to three different IV caffeine doses (3, 15 or 30 mg/kg) and a doubled loading dose (some outside the recommended dosage). Samples were collected on days 3, 5, and 7. Saliva was collected by vacuum aspiration, a method little used in clinical practice, and caffeine were determined by HPLC. The mean ratio of saliva-to-serum concentrations was 0.924. There was a small negative bias for predictive salivary versus serum concentrations. Also, there is better correlation between serum and saliva when concentrations are lower, but the correlation remains in higher values (> 8  $\mu\text{g}/\text{mL}$ ) unlike Khanna et al. [21].

De Wildt et al. [23] studied the correlation between saliva and serum levels of caffeine in 140 preterm infants aged from 24 to 34 weeks GA. They compared samples (analyzed by HPLC) according to the method of the saliva collection (not stimulation, citric acid on a swab and citric acid in cheek). They suggested that saliva correlation would be better if the saliva secretion was first stimulated by citric acid, but saliva collection stimulation would be impractical in clinical practice. In addition, the volumes collected in our study (10  $\mu\text{L}$ ) allowed us to make several determinations of each of them, showing that it is not necessary to use salivary stimulants. In this study there was also a slightly better correlation

between serum and saliva when concentrations were lower. Dobson et al. [26] reported a study of paired salivary and plasma caffeine samples in 29 preterm infants (mean GA  $27.9 \pm 2.1$  weeks). Salivary samples were obtained using a commercially available salivary collection system when they were already stable and had reached a maturity of PMA of 33–36 weeks, when they probably no longer need analyses of caffeine level in clinical practice because of the limited clinical impact of the AOP. Their correlation coefficient between saliva and blood samples was  $r = 0.87$ . Chaabane et al.[24] published a study in 13 newborns of mean GA  $32.2 \pm 0.7$  weeks not diagnosed of AOP (neonates with AOP were excluded). Each patient received 5, 10, 15, 20 and 25 mg/kg/day of IV caffeine from the first to the fifth day of birth. Samples were analyzed by enzyme multiplied immunoassay technique (EMIT). Caffeine concentrations showed a good coefficient of determination,  $r^2 = 0.76$ . Mean difference concentrations were also better when the values were smaller (Table 2) but correlation remains in higher values. They showed that saliva caffeine monitoring by EMIT could be a valid alternative to serum in preterm infants, but not in a representative population of daily practice.

The strengths of our study are a large representative sample of a target population that benefits of this drug every day, which includes extremely preterm infants who needs IV administration and several others concomitant drug. The non-invasive collection system without salivary stimulants and the caffeine doses used according the data sheet allows the reproduction of our study to increase awareness of this widely used drug. Moreover, although the correlation is better at low doses, as in most studies, the correlation remains at higher values, which makes it useful in clinical practice before making changes to the dosage. We are also aware of its limitations. The technique used to collect samples requires a few minutes. The analysis technique is currently a research technique, however, will be possible to translate it to daily practice after its simplifying in future studies. When caffeine concentrations are needed for clinical management, normally when clinicians are concerned about subtherapeutic or toxic concentrations, they are measured in blood, but our study prove that salivary sampling may be a valid non-invasive alternative that could be used not only in these cases, but more commonly to individualize and optimize the dose of this drug until we have more data on its polymorphisms that allow us to better individualize the treatment to implement optimal precision medicine in this vulnerable population. This is especially useful when is applied to extremely low birth weight infants, in whom blood sampling must be severely restricted or when doses are raised above that established in the data sheet.

## List Of Abbreviations

Apnoea of prematurity (AOP)

Gestational age (GA)

High pressure liquid chromatography (HPLC)

Intravenous (IV)

Orally (O)

Postmenstrual age (PMA)

## Declarations

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## Tables

**Table 1:** Subject demographics.

	<b>n = 47</b>
Gestational Age(weeks) +	28 (26, 30)
Birth weight (kg)*	1,11 (0,4)
Male, n (%)	30 (75 %)
Female, n (%)	17 (25 %)
Prenatal corticosteroids, n (%)	44 (95,65 %)
Caesarean birth, n (%)	35 (76,09 %)
Weight (g) *	1,26 (0,43)
Stature (cm) *	35,92 (5,75)
Head Circumference (cm) *	24,87 (5,68)
Apgar (1 min)+	7 (6, 9)
Apgar (5 min)+	9 (9, 10)
Apgar (10 min)+	10 (9, 1 0)
Surfactant, n (%)	25 (54,35 %)
*Mean (SD)	
+Median (1 <sup>st</sup> , 3 <sup>rd</sup> Q.)	

**Table 2:** Comparative table of most important caffeine salivary-serum studies.

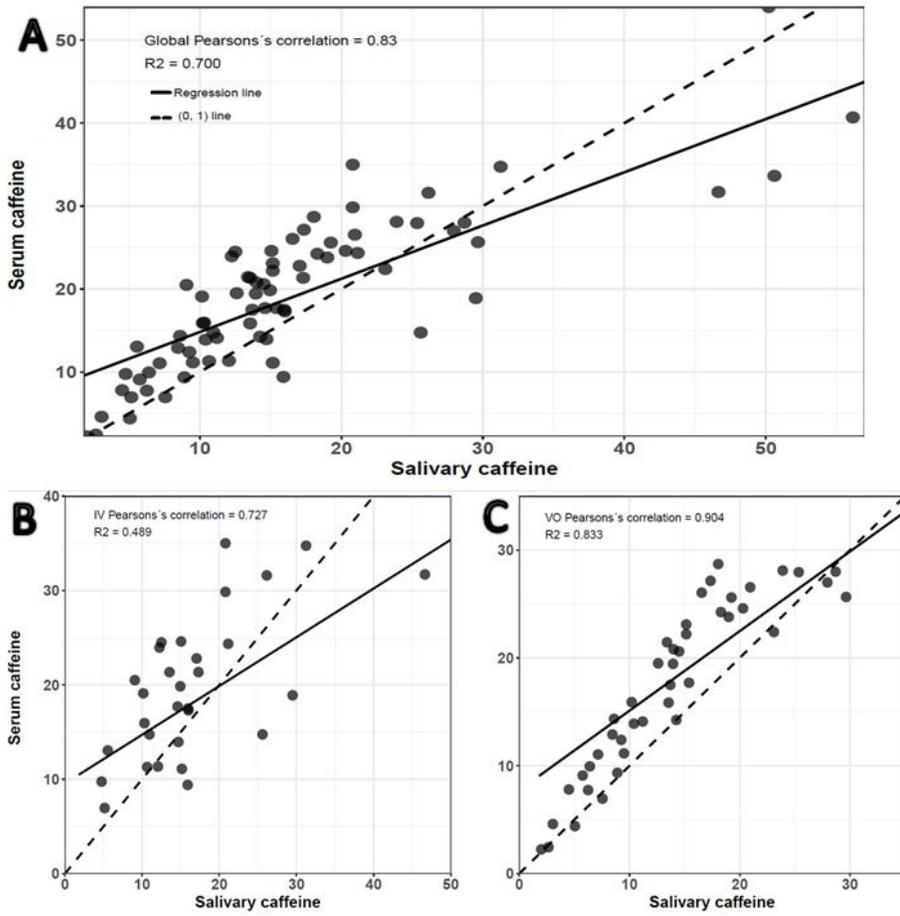
Study	N	Route	Serum to saliva ratio	R (Pearson)	R2	Mean ratio saliva-serum	Range mg/L serum	Range mg/L saliva	Mean mg/L serum	Mean mg/L saliva	Mean difference plasma and salivary concentrations	95% limits agreement
<b>OUR study</b>	47	IV/VO	1.18	0.83	0.70	0.848	1.90-53.90	2.20-56.90	19.30	16.36	-2.92	-15.09, 9.24
	20	IV	1.25	0.727	0.489	0.801			19.61	15.70	-2.51	-17.81, 12.76
	27	VO	1.15	0.904	0.833	0.866			19.16	16.6	-3.21	-12.70, 6.28
<b>Khanna et al [21]</b>	7	VO	1.40 (sd=0.61)*	0.84								
<b>Lee et al [22]</b>	59	IV				0.924	0.28-93.3	0.35-91.5	29.9	27.7		
<b>De Wildt [23]</b>	38	IV		0.68 (no estim)		0.70	3.7-22.8	1.5-16.1	13.4	9.1		
	61	IV		0.79 (citric swab)		0.70	3.3-23.6	2.1-21.2	13.4	9.2		
	41	IV		0.89 (citric cheek)		0.71	5-24.6	3.3-18	13.7	9.8		
<b>Dobson et al [26]</b>	29	VO		0.87			9.5-54.1				-0.18	-9.36, 9.00
<b>Chaabane et al [24]</b>	13	IV		0.87**	0.76		1.2-38.8	0.4-36.8			-2.5 #	-9.8, 9.5 #
- Dose 5 mg	13	IV							4.01	4.09		
- Dose 10 mg	13	IV							8.92	8.13		
- Dose 15 mg	13	IV							15.56	10.66		
- Dose 20 mg	13	IV							23.38	20.04		
- Dose 25 mg	13	IV							27.81	23.79		

\*only serum values >2 mg/L

\*\* calculated from  $\sqrt{0.76}$

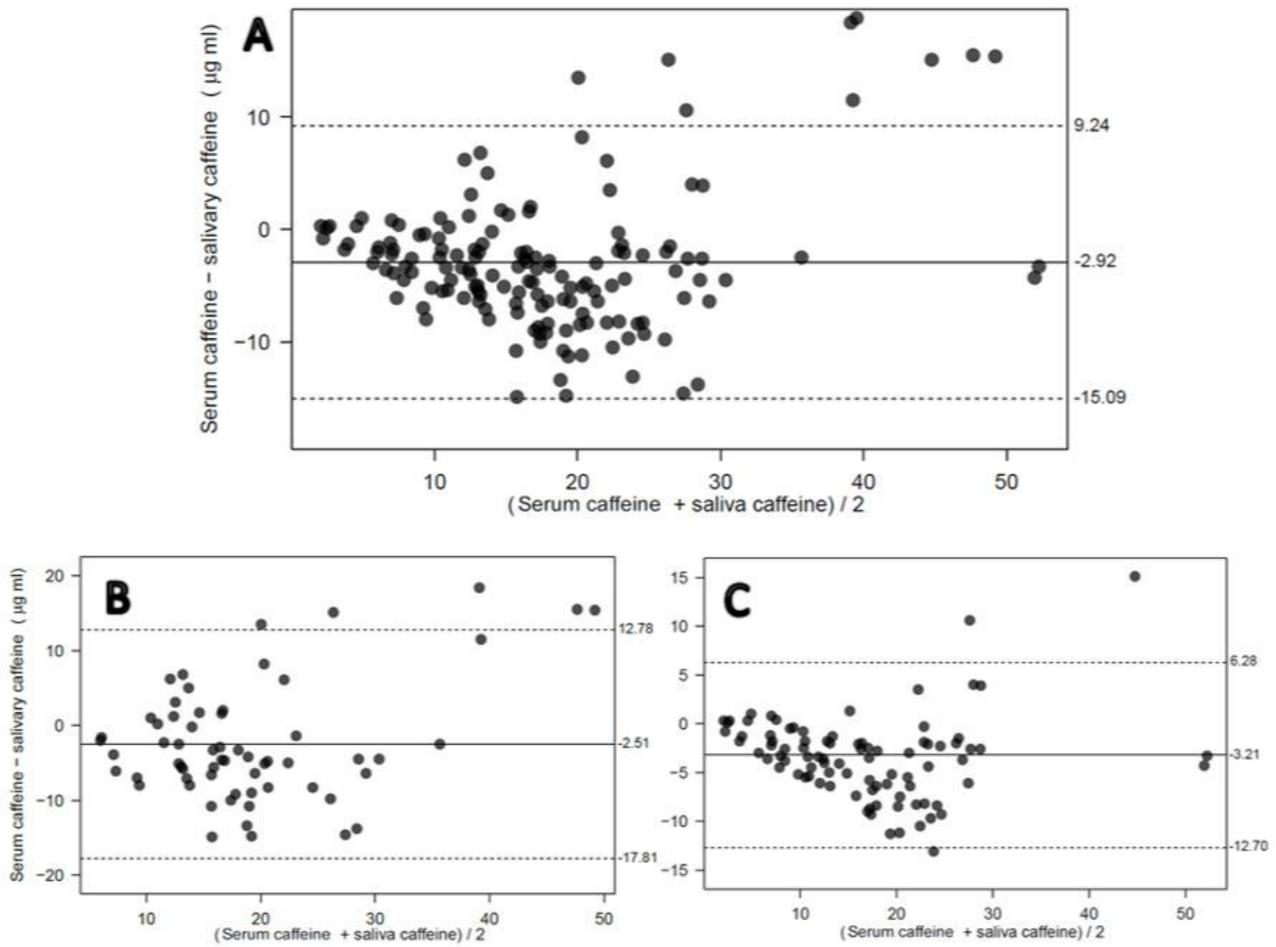
# approximate values. They were extracted from a graph.

## Figures



**Figure 1**

Salivary caffeine and plasma caffeine Scatterplot. Solid line represents regression line and dashed represent 0 intercept 1 slope line. A. Intravenous and oral administration. B. Only intravenous administration. C. Only oral administration.



**Figure 2**

Bland Altman plot for all patients. Vertical axis represents the difference in plasma and salivary concentrations by the average of the two concentrations, and 95% limits of agreements. A. Intravenous and oral administration. B. Only intravenous administration. C. Only oral administration.

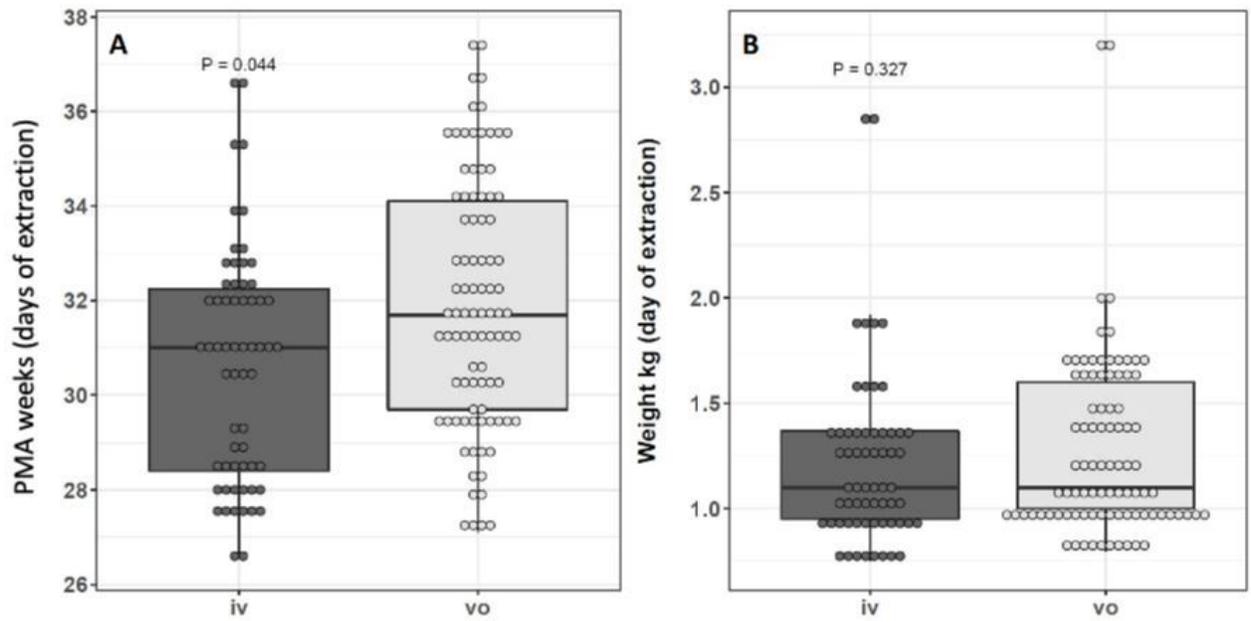


Figure 3

a) PMA at the moment of extraction levels. b) Weight in kg at the moment of extraction levels.