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Short communication

The paraxanthine:caffeine ratio in serum or in saliva as a measure of CYP1A2 activity: when should the sample be obtained?

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The human hepatic cytochrome P450 isozyme, CYP1A2, is involved in the metabolism of several drugs, including caffeine, theophylline, phenacetin, imipramine, clozapine and tacrine. Of these compounds, caffeine is the most common probe used to monitor CYP1A2 activity. Systemic caffeine clearance, caffeine N_3-demethylation clearance, the ^{13}C-caffeine breath test, and various caffeine urinary metabolic ratios have been employed as putative markers of CYP1A2 activity. As CYP1A2 most probably accounts for approximately 95% of the primary systemic caffeine clearance and possibly even more of the N_3-demethylation clearance, systemic caffeine clearance and N_3-demethylation clearance are held to be the gold standards for measurements of CYP1A2 activity (Kalow & Tang, 1993). The ^{13}C-caffeine breath test is based upon the pulmonary excretion of $^{13}CO_2$ out of a labelled caffeine dose and is dependent on physical activity and diet (Lambert *et al.*, 1983). The correlation has been shown to be low between several of the suggested urinary caffeine ratios and systemic caffeine clearance (Fuhr & Rost, 1994; Jeppesen *et al.*, 1996) and also between the suggested caffeine ratios with another (Notarianni *et al.*, 1995). Moreover, the amount of caffeine recovered in urine depends on the urinary flow (Tang *et al.*, 1994). As both the ^{13}C-caffeine breath test and the urinary metabolic ratios have their shortcomings, and as caffeine clearance measurements are time consuming and not suitable for use in large groups, the paraxanthine: caffeine test in plasma and in saliva has been developed as a promising alternative (Fuhr & Rost, 1994). However, no studies have systematically and prospectively investigated the changes in the paraxanthine:caffeine ratios in saliva and serum over time and correlated them to systemic caffeine clearance. In order to find the optimal sampling time when employing the paraxanthine:caffeine test, the present study was performed.

After giving their informed consent, 12 men took part in the investigation, which was approved by the regional Ethics Committee at the University of Umeå. All participants were healthy, as assessed by medical history, physical examination, and routine blood chemistry tests. They were all extensive metabolizers of drugs catalysed by CYP2D6 and CYP2C19, non-smokers, and they had been entirely drug-free for at least 2 weeks prior to the study. Their age (mean ± SD) was 23.6 ± 2.3 years and their body weight was 74.2 ± 8.0 kg.

After an overnight fast, the individuals received a single oral dose of 200 mg caffeine (two tablets of 100 mg Koffein ACO; ACO AB, Helsingborg, Sweden) at 08.00 h. A standardized lunch meal was served at 12.00 h and a standardized dinner at 16.00 h. Otherwise, no food was allowed for the first 8 h after drug intake. Consumption of alcohol or of food or beverages containing caffeine or other methylxanthines was not allowed from 30 h before study start until the last blood sample was obtained.

Venous blood samples (10 ml) and salivary samples (3 ml) were collected at 0, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12,

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24 and 32 h after caffeine intake. Serum was separated within 30 min. Serum and saliva were stored at −20 °C until analysis. Caffeine and paraxanthine in saliva were analysed by a high performance liquid chromatography method described earlier (Spigset *et al.*, 1998). At concentrations of 15 μmol/l, the intra-assay and interassay coefficients of variation in serum were 1.9% and 10.9%, respectively, for caffeine, and 5.1% and 12.1%, respectively, for paraxanthine. The corresponding values in saliva at 10 μmol/l were 1.8% and 4.0%, respectively, for caffeine, and 2.7% and 2.9%, respectively, for paraxanthine. The limit of quantification was 0.5 μmol/l, and the method was linear at least up to 250 μmol/l.

Pharmacokinetic parameters were calculated by use of the program package Siphar/Win, version 1.13 (SIMED SA, Creteil, France). Areas under the serum concentration-time curve (AUC) for caffeine were calculated by use of the linear trapezoidal rule with extrapolation to infinity, and caffeine clearance was calculated as dose/AUC, assuming complete absorption. For comparative statistics, Spearman's rank correlation test and the likelihood ratio test were used. The study was designed to reveal correlation coefficients of 0.6 or higher with $\alpha = 0.05$ and $\beta = 0.20$, which requires a total of 12 individuals. *P*-values < 0.05 were considered as statistically significant.

Serum concentrations of caffeine ranged from < 0.5–30.1 μmol/l and salivary concentrations of caffeine ranged from < 0.5–22.9 μmol/l. For paraxanthine, the corresponding ranges were < 0.5–8.7 μmol/l and < 0.5–6.4 μmol/l, respectively. After 32 h, concentrations of caffeine were below the limit of quantification in all but two subjects. The paraxanthine:caffeine ratio increased with time after caffeine intake both in serum (Fig. 1) and in saliva (Fig. 2), and the median ratios were nearly parallel.

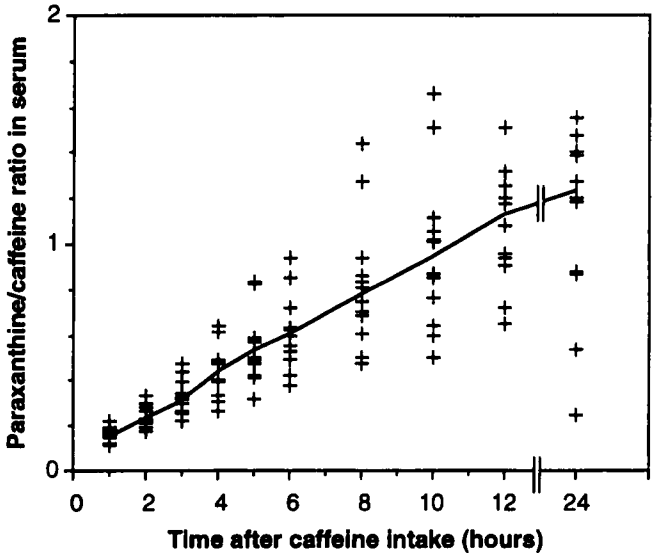

Fig. 1. Relationship between sampling time and individual paraxanthine:caffeine ratio in serum after intake of a single oral dose of 200 mg caffeine in 12 healthy men. The line shown is drawn between the median values of each sampling time. For clarity, two individual values, 2.07 after 12 h and 4.57 after 24 h, are excluded from the figure.

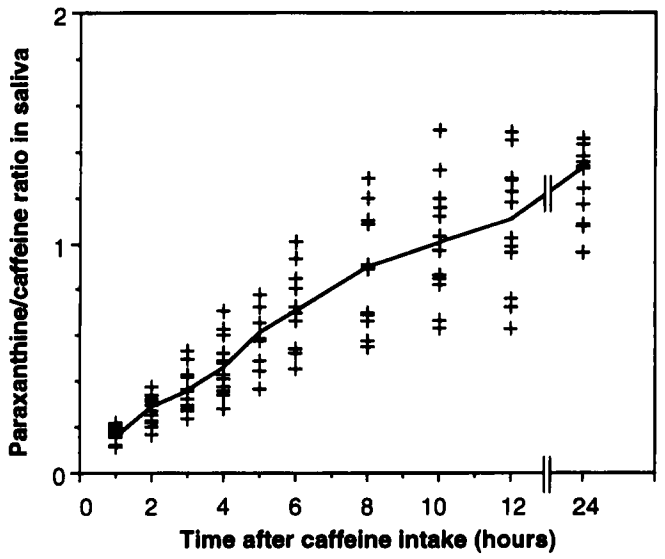

Fig. 2. Relationship between sampling time and individual paraxanthine:caffeine ratio in saliva after intake of a single oral dose of 200 mg caffeine in 12 healthy men. The line shown is drawn between the median values of each sampling time.

In general, r_s values for the correlations between caffeine clearance and the paraxanthine:caffeine ratios in serum and saliva 3–12 h after caffeine intake were high and were all statistically significant (Fig. 3). The best correlations ($r_s > 0.80$) between the paraxanthine:caffeine ratio in serum and caffeine clearance were found 3–6 h after caffeine intake. In contrast, the best correlations ($r_s > 0.80$) between the paraxanthine:caffeine ratio in saliva and caffeine clearance were found 6–10 h after caffeine intake. However, the differences between the r_s values in serum and in saliva 3–10 h after caffeine intake were not statistically significant.

When correlated against caffeine clearance per kg body weight instead of clearance, the r_s values were generally 0.01–0.06 U lower both in serum and in saliva. The correlation between the paraxanthine: caffeine ratio in serum and the corresponding ratio in saliva was above 0.9 at all sampling times 2–12 h after caffeine intake (Fig. 3).

In all individuals, concentrations of both caffeine and paraxanthine were higher in serum than in saliva. Correlations between pharmacokinetic parameters of caffeine based upon analyses in serum and in saliva, respectively, are presented in Table 1. The distribution of

Table 1. Pharmacokinetic parameters for caffeine based on serum and salivary concentrations, and r_s values for the correlation between them (n = 12)

	Serum *Median (range)*	*Saliva* *Median (range)*	r_s *value*
AUC (h·μmol/l)	169 (131–268)	130 (87–172)	0.85
Oral clearance (ml/min)	101 (64–131)	117 (99–196)	0.85
C_{max} (μmol/l)	24.4 (17.7–30.1)	17.9 (13.9–22.9)	0.54
$t_{1/2}$ (h)	5.9 (2.7–8.0)	6.8 (4.1–9.7)	0.20

AUC, area under the concentration-time curve; C_{max}, maximum concentration observed; $t_{1/2}$, elimination half-life.

the paraxanthine:caffeine ratios at the suggested optimal sampling times are shown in Table 2. Salivary concentrations of caffeine were checked in the first hours after caffeine intake in order to explore possible gingival contamination. However, the salivary concentrations were not higher compared with the serum concentrations the first hours after intake than later.

In a previous study (Fuhr & Rost, 1994), paraxanthine:caffeine ratios were calculated 3, 5 and 6 h after caffeine intake in plasma and 3, 5, 6 and 7 h after caffeine intake in saliva. In that study, the r-values were generally somewhat higher than in the present study, at least after 5, 6 and 7 h, but the study yielded inconsistent results regarding the optimal sampling time. The present study, in which salivary and serum concentrations were quantified prospectively during a prolonged time interval, indicates that the sample can be obtained without any loss of reliability up to 10 h, at least in saliva, and down to 3–4 h, at least in serum. As only non-smokers were included in the present study, a possible differential effect on caffeine clearance and on the caffeine/paraxanthine ratio by CYP1A1 induction (Schweikl *et al.*, 1993) is excluded. On the other hand, it cannot be completely excluded that other methodological factors unique to the present study, such as a meal after 8 h, might have influenced the results.

We found that the median AUC value for caffeine based upon salivary concentrations was 72% of that based upon serum concentrations, with variations of 56% to 95% between individuals. This result is in accordance with the findings in two other studies in which the mean caffeine serum to saliva ratios were 71% in premature infants (Khanna *et al.*, 1980), and 76% in adults (Soto *et al.*, 1994), respectively. Consequently, calculations based upon salivary concentrations of caffeine underestimated AUC and C_{max}, whereas the oral clearance was overestimated (Table 1). The large variability between subjects in the salivary to serum caffeine concentration ratios may explain the relatively low

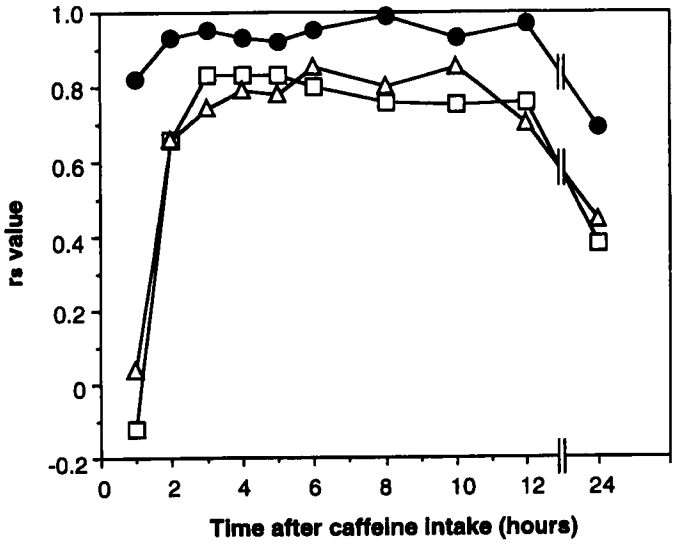

Fig. 3. Spearman rank correlation coefficients (r_s values) for the correlations between caffeine clearance and the paraxanthine:caffeine ratio in serum (open squares) and saliva (open triangles) at various points of time after intake of a single oral dose of 200 mg caffeine in 12 healthy men. Correlations between the paraxanthine/caffeine ratios in serum and in saliva at the same points of time are also shown (filled circles).

Table 2. Paraxanthine:caffeine ratios in serum and saliva 3–10 h after intake of 200 mg caffeine

	Median (range)	*Mean ± SD*
Serum (3 h)	0.32 (0.22–0.47)	0.34 ± 0.08
Serum (4 h)	0.40 (0.27–0.64)	0.43 ± 0.11
Serum (5 h)	0.50 (0.31–0.83)	0.54 ± 0.16
Serum (6 h)	0.57 (0.38–0.94)	0.59 ± 0.18
Serum (8 h)	0.78 (0.47–1.44)	0.82 ± 0.29
Serum (10 h)	0.94 (0.50–1.66)	0.97 ± 0.35
Saliva (3 h)	0.36 (0.23–0.54)	0.36 ± 0.09
Saliva (4 h)	0.45 (0.28–0.71)	0.47 ± 0.13
Saliva (5 h)	0.58 (0.37–0.78)	0.55 ± 0.13
Saliva (6 h)	0.70 (0.45–1.01)	0.70 ± 0.18
Saliva (8 h)	0.90 (0.55–1.28)	0.88 ± 0.25
Saliva (10 h)	1.00 (0.61–1.49)	1.02 ± 0.28

r-value for the correlation between caffeine AUC in serum and in saliva of 0.85. This variability might have been caused by the relatively large interindividual differences in caffeine binding to plasma proteins (Campbell *et al.*, 1987).

Calculation of caffeine clearance based upon salivary samples yielded approximately the same degree of accuracy related to systemic caffeine clearance ($r = 0.85$) as did measurements of the paraxanthine:caffeine ratios in serum or saliva 3–10 h after caffeine intake ($r = 0.74$–0.85). Thus, as it is more convenient, a single salivary or serum sample from which the paraxanthine:caffeine ratio is calculated might be the preferable method. Due to the non-linear kinetics of caffeine, the ratios calculated and the optimal sampling times suggested on the basis of the results in the present study may not necessarily be valid when a caffeine dose other than 200 mg is given.

In conclusion, this study demonstrates that the correlations between the paraxanthine:caffeine ratio in serum or in saliva and systemic caffeine clearance generally are high. Further evidence that these ratios are more reliable markers of CYP1A2 activity than the suggested caffeine urinary metabolic ratios has thus been provided, albeit indirectly. Based on the present study, the time interval from 3 to 10 h after caffeine intake seems to be optimal. Specifically, although the differences were not statistically significant, sampling 3–6 h after caffeine intake might be preferable in serum, whereas sampling 6–10 h after caffeine intake might be preferable in saliva. Given the pronounced increase in the paraxanthine:caffeine ratio with time both in serum and in saliva, the time interval from intake of caffeine to sampling should be held strictly constant among individuals in the same study.

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