CYP1A2 IN A SMOKING AND A NON-SMOKING POPULATION: CORRELATION OF URINARY AND SALIVARY PHENOTYPIC RATIOS

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SUMMARY

The use of caffeine as a probe for CYP1A2 phenotyping has been extensively investigated over the last 25 years. Numerous metabolic ratios have been employed and various biological fluids analysed for caffeine and its metabolites. These investigations have used non-smoking, smoking and numerous disease populations to investigate the role of CYP1A2 in possible disease aetiology and for induction and inhibition studies *in vivo* using dietary, environmental and pharmaceutical compounds. This investigation found that the 17X/137X CYP1A2 metabolic ratio in a 5 h saliva sample and 0-5 h urine collection was not normally distributed in both a non-smoking and a smoking population. The urinary and salivary CYP1A2 metabolic ratio was log normally distributed in the non-smoking population but the smoking population showed a bi- (or tri-)modal

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distribution on log transformation of both the urinary and salivary CYP1A2 metabolic ratios. The CYP1A2 metabolic ratios were significantly higher in the smoking population compared to the non-smoking population when both the urinary and salivary CYP1A2 metabolic ratios were analysed. These results indicate that urinary flow rate was not a factor in the variation in CYP1A2 phenotype in the non-smoking and smoking populations studied here. The increased CYP1A2 activity in the smoking population was probably due to induction of the CYP1A2 gene via the Ah receptor causing an increase in the concentration of CYP1A2 protein.

KEY WORDS

CYP1A2, phenotype, caffeine biotransformation, induction, smoking

INTRODUCTION

Human hepatic CYP1A2 undertakes a pivotal role in the metabolic biotransformation of a number of drugs, including caffeine (137X), theophylline /1/, imipramine /2/, as well as the bioactivation of procarcinogens /3/ and heterocyclic amines /4/. There has been found to be a wide variation in CYP1A2 activity between individuals, which may be due to induction/inhibition of CYP1A2 activity by environmental or dietary compounds /5/. To complicate the matter further a polymorphism in CYP1A2 activity has been suggested /6,7/. However, these observations have been the subject of an extensive and conflicting debate in the literature /8/.

The oxidative metabolism of 1,3,7-trimethylxanthine (137X) or caffeine in man is complex /9/ with up to nine enzymes involved in its metabolism /10/. Of these various metabolic biotransformations, 137X N-3 demethylation is the most predominant and accounts for 80% of systemic 137X clearance /11,12/. The N-3 demethylation of 137X is mediated almost exclusively by CYP1A2 (the contribution of other CYPs to this biotransformation is negligible). Thus both the systemic clearance and N-3 demethylation partial biotransformation of 137X can serve as a biomarker of CYP1A2 activity.

To date several urinary metabolic ratios (UMR) have been proposed to reflect CYP1A2 activity /8,13/. However, these various

UMRs based on secondary or tertiary metabolites not formed exclusively by CYP1A2 do not accurately reflect CYP1A2 activity. The other simplified UMR can vary dramatically with urine flow, interethnic differences in renal function and different sampling protocols /8,13/. To date the simple 17X (1,7-dimethylxanthine, paraxanthine)/137X plasma or saliva metabolic ratio has been proposed as the best parameter to assess CYP1A2 activity /1/.

The present study was designed to investigate the relationship between the 17X/137X CYP1A2 metabolic ratio in a 5 h saliva sample and 0-5 h urine collection. The two populations under investigation were a non-smoking and a smoking cohort of British Caucasians.

MATERIALS AND METHODS

Chemicals

1,3,7-Trimethylxanthine (137X, caffeine), 3,7-dimethylxanthine (37X, theobromine), 1,7-dimethylxanthine (17X, paraxanthine), 1,3-dimethylxanthine (13X, theophylline), 1-methylxanthine (1X), 3-methylxanthine (3X), 7-methylxanthine (7X), 3,7-dimethyluric acid (37U), 1,3-dimethyluric acid (13U), 1,7-dimethyluric acid (17U), 1,3,7-trimethyluric acid (137U), β-hydroxyethyltheophylline (BHET), paracetamol, analar grade chloroform, isopropanol and ammonium sulphate were purchased from the Sigma-Aldrich Chemical Company (Poole, Dorset, UK), high performance liquid chromatography (hplc) grade methanol was from Rathburn Chemicals (Wakerburn, Scotland, UK). A Spherisorb 5μ 250 x 4.6 mm ID C_{18} column and Security Spherisorb 10μ C_{18} guard column were purchased from Phenomenex (Macclesfield, Cheshire, UK). C_{18} solid phase extraction (SPE) columns were obtained from Superlco Sigma-Aldrich Chemical Company (Poole, Dorset, UK).

Volunteers

One hundred and fifty staff and students at the Faculty of Applied Sciences, University of the West of England, Bristol, were recruited for the investigation. All volunteers were not taking any medication (including the oral contraceptive pill). None of the participants was

known to have significantly impaired renal or hepatic function. This investigation was approved by the ethics committee of the University of the West of England and all volunteers gave informed written consent.

Volunteer descriptions

- One hundred non-smoking volunteers (55 male, 45 female), mean age 28.4 ± 8.3 years, range 18-45 years;
- Fifty smoking volunteers (20 male, 30 female), mean age 29.1 ± 6.7 years, range 18-50 years.

CYP1A2 phenotyping

Foods and drinks containing caffeine, such as chocolate, coca-cola, tea and coffee, were not allowed from 12 h prior to the investigation until the study was complete. Individuals were also asked not to consume grapefruit or orange juice during the study.

Following an overnight fast, subjects emptied their bladders and consumed 4 x 50 mg 'Pro-Plus' tablets (Roche PLC), equivalent to 200 mg 137X, at 09.00 h. Each individual then collected all urine voided until 14.00 h. A 2 ml saliva sample was also collected at 14.00 h from each subject.

Analysis

Biological specimens were subjected to liquid-liquid extraction for urine samples /6/ and solid-phase extraction for salivary samples /6/ before undergoing analysis by reversed phase hplc with UV detection /6/.

Statistical analysis

Data analyses were performed using SPSS 10.0 by Student's t-test and Wilcoxon rank sum test. A value of $p < 0.05$ was taken to indicate statistical significance.

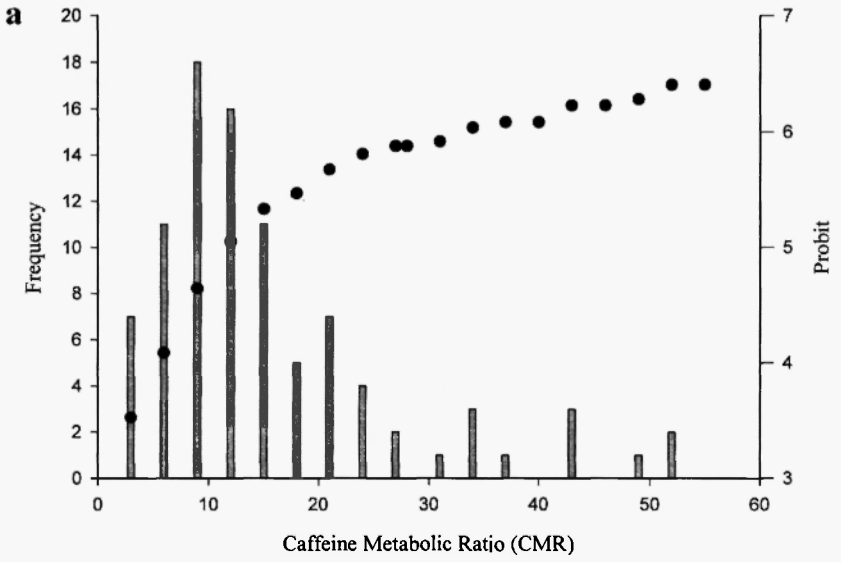
RESULTS

There was no significant difference in mean age (± SD) between the non-smoking and smoking volunteers ($p > 0.05$, Student's t-test), nor were there any significant differences between male and female volunteers in mean age (± SD) both within and between the non-smoking and smoking volunteers (results not shown).

The frequency distribution and normal probability (probit) plots for the CYP1A2 urinary metabolic ratio in a non-smoking population can be seen in Figure 1a. The data were highly skewed to the right and showed non-linear probit analysis indicating multi-modality. When the CYP1A2 urinary metabolic ratio was $\log_{10}$ transformed then the frequency distribution and probit plots showed that the CYP1A2 urinary metabolic ratio was in fact a log normal distribution (Fig. 1b). The smoking population frequency distribution for CYP1A2 urinary metabolic ratios can be seen in Figure 2a. The data were not normally distributed as indicated by probit plot analysis. Log_{10} transformation of the CYP1A2 urinary metabolic ratios, as seen in Figure 2b, indicated that the CYP1A2 urinary metabolic ratios were possibly trimodally or bimodally distributed. The CYP1A2 metabolic ratios in the smoking population were significantly higher than for the non-smoking population (Table 1, $p < 0.05$, Wilcoxon rank sum test).

The data for the salivary CYP1A2 metabolic ratio frequency distribution and probit analysis in a non-smoking population can be seen in Figure 3a. The data were highly skewed and not normally distributed. Log_{10} transformation of the CYP1A2 metabolic ratio resulted in the data showing a log normal distribution (Fig. 3b). When the salivary CYP1A2 metabolic ratio frequency distribution and probit analysis in a smoking population was investigated (Fig. 4a), the data were not normally distributed as indicated by the probit plot analysis. Log_{10} transformation of the CYP 1A2 metabolic ratio resulted in the data remaining as a trimodally or bimodally distributed population, as indicated by the probit plot (Fig. 4b). The salivary CYP1A2 metabolic ratios in the smoking population were significantly higher than for the non-smoking population (Table 1, $p < 0.05$, Wilcoxon rank sum test).

The log normal frequency distribution for both the urinary and salivary CYP1A2 metabolic ratio in a smoking and a non-smoking population showed that both phenotyping investigations (urinary and salivary) resulted in the smoking population having a higher median



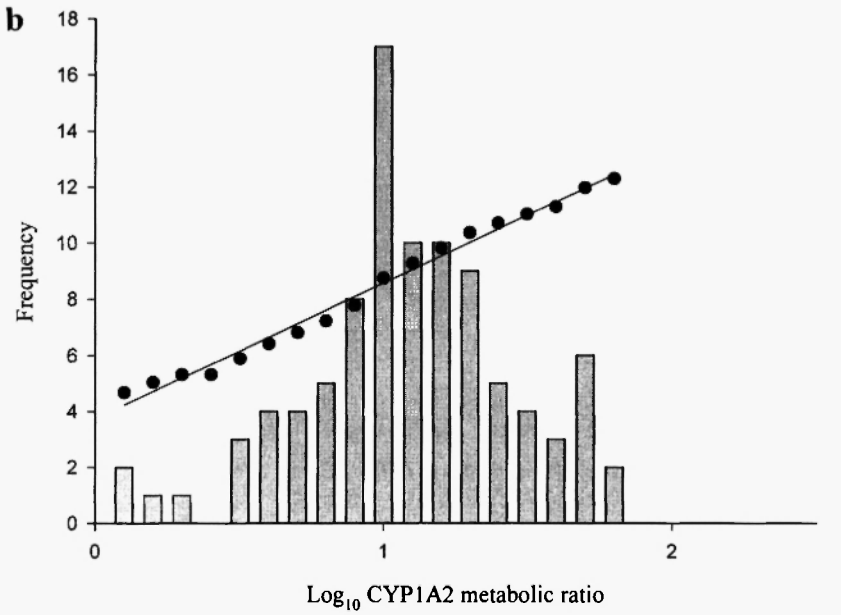


Fig. 1: Urinary CYP1A2 metabolic ratios in a non-smoking population, n = 100. **a.** probit plot and frequency distribution; **b.** probit plot and $\log_{10}$ frequency distribution.

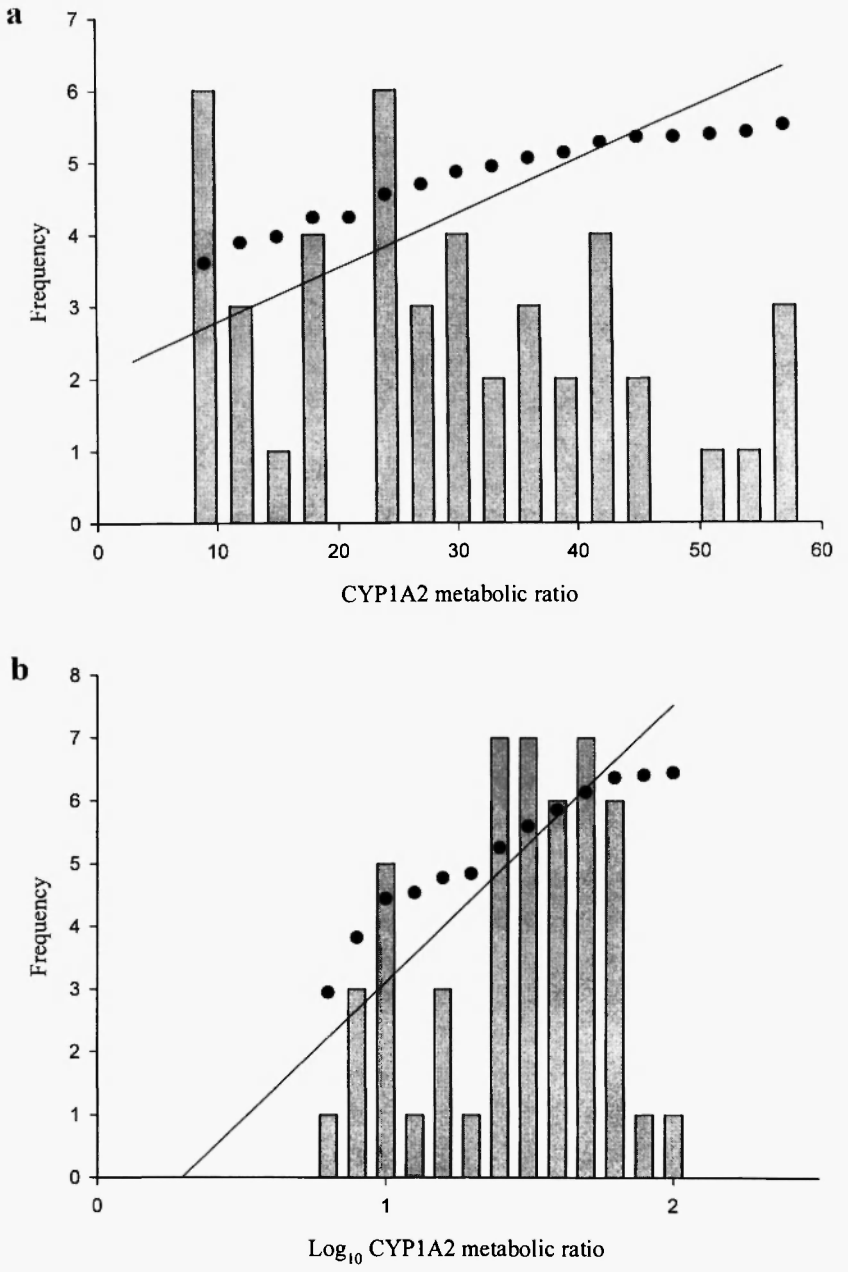


Fig. 2: Urinary CYP1A2 metabolic ratios in a smoking population, n = 50. **a.** probit plot and frequency distribution; **b.** probit plot and $\log_{10}$ frequency distribution.

TABLE 1

Statistical transformations of urinary and salivary CYP1A2 metabolic ratios in a smoking (n = 100) and a non-smoking (n = 50) population

Population	n	Mean ($\log_{10}$) CYP1A2 ratio	± SD	Median CYP1A2 ratio	Range
Non-smoking (urinary data)	100	1.10	0.56	11.20	0.81-101.00
Non-smoking (salivary data)	100	1.03	0.42	10.50	0.97-94.40
Smoking (urinary data)	50	1.41	0.31	27.80*	6.12-105.80
Smoking (salivary data)	50	1.35	0.29	22.24*	5.42-84.62

* $p < 0.05$, Wilcoxon rank sum test.

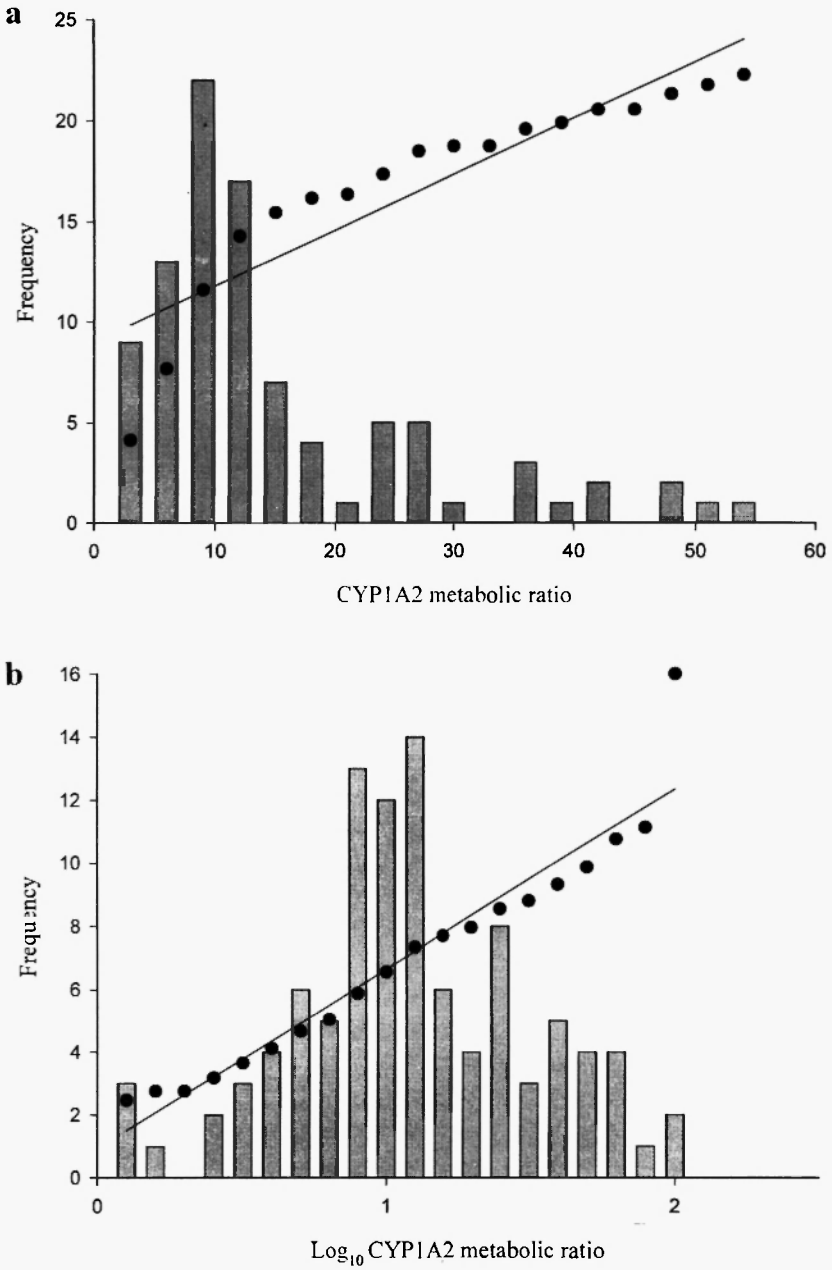


Fig. 3: Salivary CYP1A2 metabolic ratios in a non-smoking population, n = 100. **a.** probit plot and frequency distribution; **b.** probit plot and $\log_{10}$ frequency distribution.

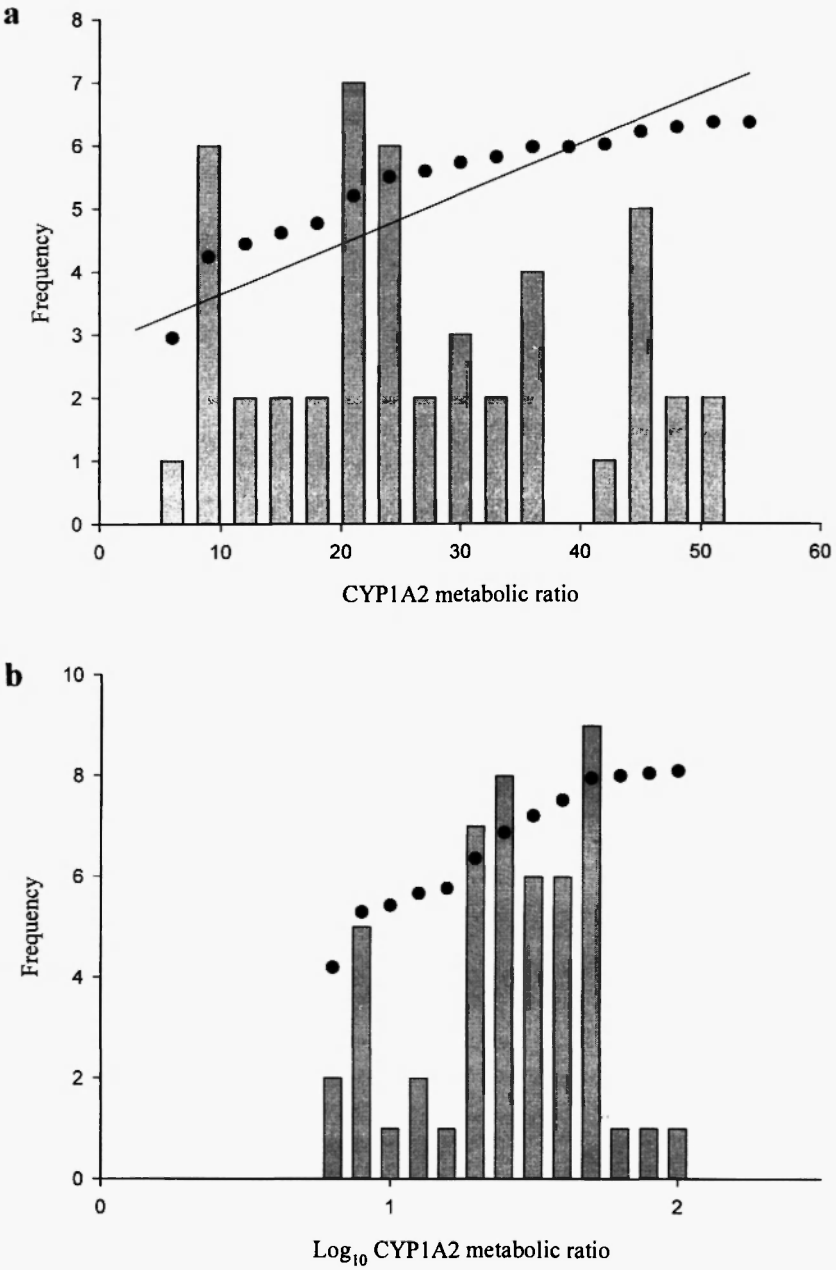
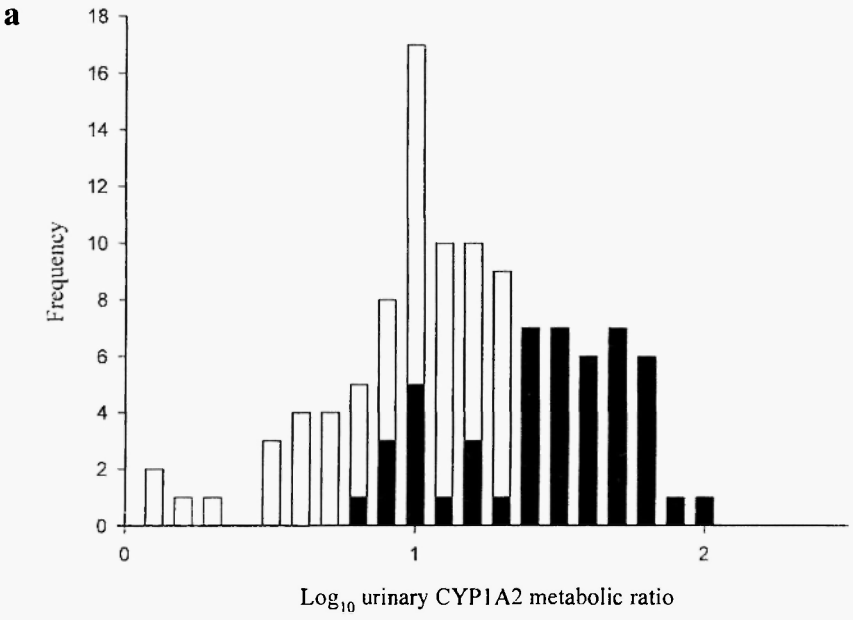


Fig. 4: Salivary CYP1A2 metabolic ratios in a smoking population, n = 50. **a.** probit plot and frequency distribution; **b.** probit plot and $\log_{10}$ frequency distribution.

CYP1A2 metabolic ratio than the non-smoking population (Fig. 5, Table 1, $p < 0.05$, Wilcoxon rank sum test). There was no significant difference between the urinary and salivary $\log_{10}$ CYP1A2 metabolic ratio in the non-smoking population (Table 1, $p > 0.05$, Student's t-test), nor were there any significant differences in the urinary and salivary median CYP1A2 metabolic ratio in the smoking population (Table 1, $p > 0.05$, Wilcoxon rank sum test).

DISCUSSION

The results from the investigation of urinary and salivary CYP1A2 metabolic ratios in a non-smoking population indicate that the phenotypes showed a log normal distribution (Figs. 1b and 3b). No significant difference was found between mean $\log_{10}$ CYP1A2 urinary and salivary ratios or median urinary and salivary ratios (Table 1). This is of interest since some reports using these metabolic ratios have suggested that CYP1A2 activity is trimodally distributed /3,14/. The use of the various metabolic ratios with urinary analysis has been questioned by a number of research groups /8/. The main concern with the use of urinary collection for the analysis of CYP1A2 derived metabolites are that urine flow is a significant factor in determining the detection of a polymorphism in CYP1A2 activity. Thus it appears that the variances and heterogeneity of urine flow and the effects that these factors have on the CYP1A2 metabolic ratio chosen for this investigation are intrinsically linked /12/. These factors concerning urinary flow rate could explain the bimodal results seen in the non-smoking population (Fig. 1a). However, other groups have reported that CYP1A2 activity is log-normally distributed in man /13,15/ and this can be seen in the non-smoking population in this investigation (Fig. 1b). To overcome the problems of differential urine flow on the CYP1A2 metabolic ratio, plasma or salivary CYP1A2 metabolic ratios have been investigated /1,8/. These investigations concluded that CYP1A2 metabolic ratios were better indicators of CYP1A2 activity when plasma or saliva was used as the sampling media, and not urine. This was investigated in this study. A bimodal distribution of CYP1A2 phenotypes was seen in the non-smoking population (Fig. 3a) but this disappeared on log normal transformation (Fig. 3b). There was no significant difference between the non-smoking urinary and



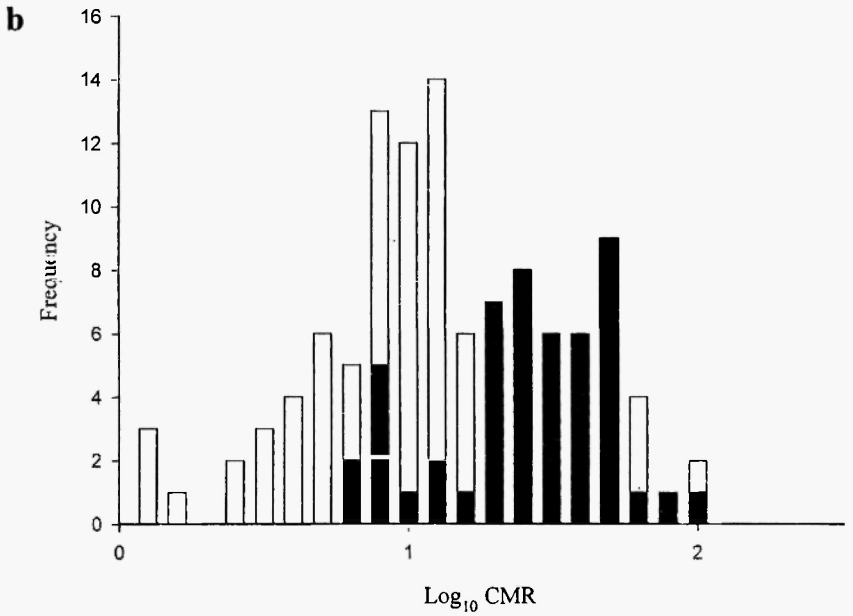


Fig. 5: Frequency distribution of **a.** urinary $\log_{10}$ CYP1A2 metabolic ratios and **b.** salivary $\log_{10}$ CYP1A2 metabolic ratios in a non-smoking (open bars) and a smoking (black bars) population.

salivary CYP1A2 metabolic ratios (Table 1, $p > 0.05$, Wilcoxon rank sum test).

The components of cigarette smoke contains many well known inducers of CYP1A2 activity /16/ and the CYP1A2 metabolic ratios found in the various phenotyping investigations are higher in smoking populations than non-smoking populations /6,13,17/. The urinary CYP1A2 metabolic ratio frequency distribution and probit plot can be seen in Figure 2a. This appears not to be normally distributed, and a log normal transformation of these data resulted in either a trimodal or bimodal distribution of overlapping subpopulations to become apparent (Fig. 2b). To see whether this could be due to variation in urinary flow rate, the same population was also investigated using the same CYP1A2 metabolic ratio but with saliva as the biological fluid analysed for caffeine and metabolites. The results of the salivary CYP1A2 phenotyping investigation can be seen in Figure 4. The CYP1A2 frequency distribution and probit plot appear not to be normally distributed (Fig. 4a). A log normal transformation of the data in Figure 4a resulted in a trimodally or bimodally distributed population with two or three overlapping subpopulations (Fig. 4b). There was no significant difference between the smoking population in terms of urinary and salivary CYP1A2 metabolic ratios (Table 1, $p > 0.05$, Wilcoxon rank sum test), indicating that differential urine flow was not a factor in this population investigation.

The smoking population, however, did have significantly higher CYP1A2 metabolic ratios than the non-smoking population when either the urinary or salivary metabolic ratios were investigated (Table 1, Fig. 5, $p < 0.05$, Wilcoxon rank sum test). The possibility that the tri- or bimodal distribution of CYP1A2 activity in the smoking population is due to genetic factors is supported by the observation that CYP1A1 activity is polymophically expressed in man due to allelic variants in the Ah receptor /18/. CYP1A2 is also induced via the Ah receptor, and the tri- or bimodal distribution seen in Figures 2b and 4b could be the result of a genetic polymorphism in the Ah receptor causing a high or low inducible response to the polyaromatic hydrocarbons in cigarette smoke.

In conclusion, the results presented in this investigation show that CYP1A2 phenotyping (using both urinary and salivary CYP1A2 metabolic ratios) resulted in significantly higher CYP1A2 metabolic ratios in a smoking population than in a non-smoking population.

These results may in turn be due to a genetic polymorphism in the Ah receptor.

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