The effect of liver disease on urine caffeine metabolite ratios

Objectives: A number of caffeine metabolite ratios (CMRs) have been proposed to measure CYP1A2 activity in vivo. The effect of liver disease on these ratios is not clear. The objective of this study was to determine the influence of liver disease on caffeine metabolite ratios.

Study design: Two studies were performed. First, in healthy control subjects and in subjects with cirrhosis, caffeine clearance was measured by intravenous infusion of stable isotope-labeled caffeine while subjects consumed oral caffeine. Second, spot urine samples were collected from control subjects and from subjects with either chronic hepatitis or cirrhosis while they consumed caffeine.

Results: In study 1, caffeine clearance was decreased in subjects with cirrhosis (control mean, 0.14 L/hr/kg; cirrhosis mean, 0.04 L/hr/kg; $p = 0.003$). CMRs were affected by liver disease (e.g., ratio characterizing paraxanthine demethylation [AAMU+1X+1U/17U], control median, 8.3; cirrhosis median, 6.2; $p = 0.005$). AAMU+1X+1U/17U correlated significantly with caffeine clearance in the control group ($r^2 = 0.68$; $p = 0.001$) and in subjects with cirrhosis ($r^2 = 0.41$; $p = 0.05$). In study 2, there was a significant difference between control subjects and subjects with cirrhosis for AAMU+1X+1U/17U (median for control subjects, 6.2; median for subjects with cirrhosis, 4.3; $p = 0.001$) but not between control subjects and patients with chronic hepatitis.

Conclusions: We conclude that AAMU+1X+1U/17U is affected by liver disease and reflects the decrease in CYP1A2 activity in subjects with cirrhosis. AAMU+1X+1U/17U measured from a spot urine sample reflects caffeine clearance in subjects with cirrhosis and may be useful as a hepatic function test. Despite the large interindividual variation observed, the test can be repeated easily in the same patient and an individual patient's decline in CYP1A2 activity, such as in patients with progressively deteriorating liver function, can be monitored. (Clin Pharmacol Ther 1996;59:624-35.)

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Caffeine metabolite ratios (CMRs) are commonly used to assess the activity in vivo of hepatic drug metabolizing enzymes, especially CYP1A2 activity, and to phenotype acetylator status. Of the many CMRs proposed to reflect CYP1A2 activity,[1-4] the ratio AAMU+1X+1U/17U (for metabolite abbreviations, see Table II) has been shown to be the ratio most closely correlated to caffeine clearance under a variety of different conditions[1,5] and therefore thought to be most robust.[6] Although the dose of caffeine[5] and previous exposure to inducers or inhibitors of CYP1A2[1,7] affect the value of this ratio, as they indeed affect the metabolism of caffeine, the effect of hepatic disease on this ratio or on other CMRs remains unknown. Caffeine metabolism is impaired in liver disease[8,9] and the measurement of caffeine clearance is used as a research tool to assess overall hepatic function in patients with cirrhosis.[10,11] A relationship has been reported between

caffeine clearance and the severity of cirrhosis as judged by the Child-Turcotte-Pugh scoring system for hepatic dysfunction.[12,13]

There are two reasons to investigate the effect of liver disease on CMRs. The first is to determine whether these ratios are affected by hepatic dysfunction and which ratio(s) best reflects CYP1A2 activity under these conditions. The second reason is to explore the possibility of the use of CMRs as a hepatic function test, particularly if their correlation to caffeine clearance is maintained in subjects with cirrhosis. Caffeine clearance has been used as a research tool to assess hepatic function in a variety of pathologic conditions. Direct measurement of caffeine clearance is unwieldy for routine clinical use, even with use of the overnight salivary clearance method.[11] Also, in the light of the wide intersubject variation in CYP1A2 activity,[6] the dose-dependency of the metabolism of caffeine,[14,15] and environmental influences on caffeine clearance, any single measurement of caffeine clearance would be difficult to interpret and would have limited value. Because ratios of metabolites in urine describe concentrations of metabolites relative to one other, they are essentially independent of total recovery of all metabolites from the urine after a particular test dose.[16] Thus, if a ratio measured from a single spot urine sample correlates closely with caffeine clearance, then one could use this ratio instead of caffeine clearance to assess hepatic dysfunction in subjects with cirrhosis. Such a simple investigation could be used in sequential fashion (as routine liver function tests are) in the same individual to provide information on the rate of progression of liver disease in that individual. Determination of which CMR would be most useful for both purposes was the primary aim of our study.

We present data from experiments exploring the effect of hepatic dysfunction on a number of proposed CMRs and correlations of CMRs to caffeine clearance (and presumably CYP1A2 activity) in healthy subjects and subjects with cirrhosis. We also examined whether these ratios could distinguish healthy controls from individuals with either chronic hepatitis or cirrhosis of varying severities.

METHODS

Correlation of caffeine clearance with CMRs in healthy control subjects and subjects with cirrhosis (experiment 1). The controls were 12 healthy men (age range, 23 to 64 years; mean age, 43 years) who regularly consumed caffeine. They were all smokers but were not taking any medication. The subjects were given 25 mg stable isotope-labeled caffeine ([2-^{13}C,1,3-$^{15}N_2$]-caffeine) by intravenous injection at 8 AM and blood was collected at 5, 15, 30, and 45 minutes and at 1, 2, 3, 5, 8, 12, and 24 hours after this dose for measurement of caffeine concentrations and clearance. Immediately after the caffeine injection, 0.5 gm/kg galactose was administered intravenously over 5 minutes and blood was collected for galactose estimation every 5 minutes for the next hour, starting at 20 minutes after the infusion was completed. During this period of 24 hours, subjects consumed multiple beverages that contained caffeine. The amount and timing of ingestion approximately matched their intake the day before, except that decaffeinated coffee or tea with added caffeine was substituted for their regular beverage. Most of the caffeine was taken in the morning. The dose of caffeine that subjects consumed ranged from 3.1 to 9.9 mg/kg/day (mean, 5.6 mg/kg/day). Urine was collected for 24 hours, and single spot urine samples were also collected separately during this time.

Eleven subjects with cirrhosis were enrolled in the study: 10 men and one woman (age range, 37 to 64 years; mean age, 48 years). Most had cirrhosis caused by either alcohol or hepatitis B or C. One had biliary cirrhosis. Their Child-Turcotte-Pugh scores ranged from 5 to 12 (mean, 8.8); eight of the 11 subjects would be classified as Child's B.[17] They were taking a variety of medications, including spironolactone and furosemide, and one subject was taking cimetidine. Nine of the 11 subjects smoked cigarettes and regularly consumed caffeine. On the first day, these subjects were given 25 mg stable isotope-labeled caffeine and blood was collected similarly to the collection for the control subjects except that the sampling continued every 12 hours for a total of 96 hours. As in the control experiment, galactose administration followed the caffeine injection, but the blood sampling, starting at 20 minutes after the infusion, occurred every 10 minutes for the next hour. Subjects who regularly consumed caffeine were given caffeine-containing beverages in the same manner as the control subjects, but for the 4 full days that the stable isotope-labeled caffeine disposition was being characterized. The average dose of caffeine was 3 mg/kg/day (range, 0.7 to 6.2 mg/kg/day). Again, most of the caffeine was consumed in the morning and the amount and timing of the oral caffeine was identical on each day. The two subjects who did not regularly consume caffeine were

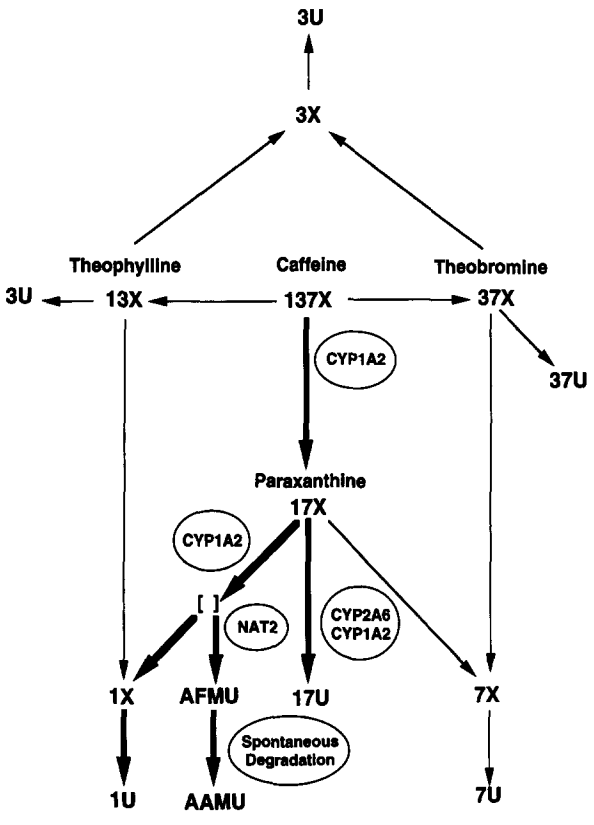

Fig. 1. Primary pathways of caffeine metabolism. *Heavy arrows* indicate major pathways; adjacent to these arrows are the enzymes thought to catalyze the pathway. 137X, 1,3,7-Trimethylxanthine (caffeine); 17X, 1,7-dimethylxanthine (paraxanthine); 37X, 3,7-dimethylxanthine (theobromine); 13X, 1,3-dimethylxanthine (theophylline); 17U, 1,7-dimethyluric acid; 13U, 1,3-dimethyluric acid; 1X, 1-methylxanthine; 3X, 3-methylxanthine; 7X, 7-methylxanthine; 1U, 1-methyluric acid; 3U, 3-methyluric acid; 7U, 7-methyluric acid; 137U, 1,3,7-trimethyluric acid; AFMU, 5-acetylamino-6-formylamino-3-methyluracil; AAMU, 5-acetylamino-6-amino-3-methyluracil.

given approximately 4 mg/kg caffeine in a single dose of decaffeinated coffee on the morning of the first day of the study and no further caffeine. Urine was collected in a manner similar to that used for the control subjects.

Caffeine metabolite ratios in subjects with different severities of liver disease (experiment 2). Spot urine samples (obtained in the afternoon or late morning) were collected from caffeine-consuming subjects who were attending hepatology outpatient clinics at San Francisco General Hospital. These subjects (age range, 28 to 69 years; mean, 50 years), had a variety of hepatic diseases but could be divided into a group of 11 with chronic hepatitis and a group of 32 with cirrhosis. Approximately 50% were nonsmokers. Nine healthy control subjects who regularly consumed caffeine were also enrolled and had spot urine samples collected. Seventy-five percent were nonsmokers. To maximize numbers for the analysis, CMRs calculated from the afternoon spot urine samples in experiment 1 were combined with the CMRs of these subjects. Some of the subjects had to be excluded (see *analytical methods*) but, for the subjects included in this analysis, approximate caffeine consumption 24 hours before the spot urine sample was collected ranged from 1.1 to 8.7 mg/kg (mean, 3.3 mg/kg). To estimate their caffeine consumption, we assigned a value, based on typical values for these beverages, of 100 mg per cup of coffee, 50 mg per cup of tea, and 40 mg per 12 ounces of a caffeine-containing soft drink.

Analytical methods. The acquisition of chemicals and assays for plasma isotope-labeled caffeine and urine caffeine concentrations are described in a previous article.[14] Metabolites of caffeine in the urine were measured with use of HPLC, and our revised method has recently been published.[5] Unfortunately, some subjects had concentrations of major metabolites in the urine that were below a level that we could reliably quantitate. Concentrations below our lowest reliable quality control value were excluded. As a result, three subjects with cirrhosis in experiment 1 could not be used in the estimation of various CMRs. However, they were still included in any analysis for caffeine clearance. An occasional other subject had to be excluded for the same reasons for some of the analyses. This is seen in some of the results from the spot urine collections. In experiment 2, a number of subjects had to be excluded for the same reasons. This left, when combined with the afternoon spot urine samples from experiment 1, 17 control subjects, eight subjects with chronic hepatitis, and 27 subjects with cirrhosis (13 subjects classified as Child's A and 14 as Child's B or C). Galactose was measured by an enzymatic assay with use of galactose dehydrogenase, NAD^+, and spectrophotometry.

Pharmacokinetic analysis. A one- or two-compartment body model was used to fit plasma concentrations of isotope labeled caffeine after intravenous injection with use of extended least-squares regression (MKMODEL, Biosoft, Milltown, N.J.). Area under the plasma concentration–time

Table I. Stable isotope-labeled caffeine pharmacokinetic parameters: experiment 1

	V_C (L/kg)*	$t_{1/2}\alpha$ (min)*	V_{SS} (L/kg)	CL (L/hr/kg)†	$t_{1/2}$ (hr)†
Control subjects					
Mean	0.20	7.1	0.65	0.14	4.2
CV (%)	33%	56%	23%	48%	44%
Subjects with cirrhosis					
Mean	0.28	6.6	0.71	0.04	21.1
CV (%)	44%	38%	26%	72%	67%

V_C, Volume of central compartment; $t_{1/2}\alpha$, distribution phase half-life; V_{SS} volume of distribution at steady state; CL, caffeine clearance; $t_{1/2}$ elimination half-life; CV, coefficient of variation.
*Two-compartment fitting was possible only in some subjects: six control subjects and six patients with cirrhosis.
†Significant difference between control subjects and subjects with cirrhosis ($p < 0.05$).

Table II. Comparison of caffeine metabolite ratios between control subjects and subjects with cirrhosis: experiment 1

No.	*Ratio*		*n*	*Mean*	*Median*	*CV (%)*
1	$\frac{AAMU+1X+1U}{17X}$ (paraxanthine demethylation ratio)	C	12	8.63	8.13	40
		L	8	7.15	4.25	155
2	$\frac{AAMU+1X+1U}{17U}$ (paraxanthine demethylation ratio)	C	12	9.98	8.28	39
		L	8	5.33*	6.23*	32
3	$\frac{17X+17U}{137X}$ (*N*-3 demethylation ratio)	C	12	8.84	8.34	34
		L	7	5.60*	6.17	34
4	$\frac{17U}{17X}$ (paraxanthine hydroxylation ratio)	C	12	0.93	0.79	47
		L	8	1.35	1.27	77
5	$\frac{AAMU}{AAMU+1X+1U}$ (acetylation ratio)	C	12	0.36	0.39	36
		L	8	0.37	0.42	36
6	$\frac{IU}{1X}$ (xanthine oxidase ratio)	C	12	1.86	1.65	28
		L	8	2.00	1.99	14
7	$\frac{(DX+DU)+2(MX+MU+AAMU)}{3(MX+MU+DX+DU+TX+TU+AAMU)}$ (total demethylation ratio)	C	12	0.58	0.59	4
		L	8	0.52*	0.53*	12
8	$\frac{17X+17U+1X+1U+7X+7U+AAMU}{MX+MU+DX+DU+TX+TU+AAMU}$ (*N*-3 demethylation ratio)	C	12	0.93	0.93	3
		L	8	0.83*	0.84*	10

Ratios calculated from 24-hour collection of urine.
MX, All monomethylxanthines; DX, all dimethylxanthines; TX, caffeine; MU, all monomethyluric acids; DU, all dimethyluric acids; TU, 1,3,7-trimethyluric acid; 1X, 1-methylxanthine; 7X, 7-methylxanthine; 17X, 1,7-dimethylxanthine (paraxanthine); 1U, 1-methyluric acid; 7U, 7-methyluric acid; 17U, 1,7-dimethyluric acid; 137X, 1,3,7-trimethylxanthine (caffeine); AAMU, 5-acetylamino-6-amino-3-methyluracil.
C, Control subjects; L, patients with liver disease.
*Significant difference between control subjects and subjects with cirrhosis by means of two sample *t* test (means) or Mann-Whitney test (medians) ($p < 0.05$).

curve (AUC) was computed by the log-linear trapezoidal rule; steady-state volume of distribution was calculated with use of area under the moment curve[18]; and clearance was calculated by division of the dose of labeled caffeine by the area under the concentration–time curve. Galactose elimination capacity (GEC) was calculated as described by Tygstrup.[19]

Table III. Correlation of caffeine clearance with caffeine metabolite ratios in subjects with cirrhosis: experiment 1

No.	Ratio	n	CL/W (L/hr/kg)		CL/LWT (L/hr/kg)	
			p	r^2	*p*	r^2
1	$\frac{AAMU+1X+1U}{17X}$ (paraxanthine demethylation ratio)	8	0.51	0	0.18	0.15
2	$\frac{AAMU+1X+1U}{17U}$ * (paraxanthine demethylation ratio)	8	0.05	0.41	0.03	0.49
3	$\frac{17X+17U}{137X}$ (*N*-3 demethylation ratio)	7	0.10	0.34	0.03	0.58
4	$\frac{17U}{17X}$ (paraxanthine hydroxylation ratio)	8	0.91	0	0.49	0
5	$\frac{AAMU}{AAMU+1X+1U}$ (acetylation ratio)	8	0.18	0.15	0.41	0
6	$\frac{1U}{1X}$ (xanthine oxidase ratio)	8	0.44	0	0.72	0
7	$\frac{(DX+DU)+2(MX+MU+AAMU)}{3(MX+MU+DX+DU+TX+TU+AAMU)}$ (total demethylation ratio)	8	0.10	0.29	0.03	0.49
8	$\frac{17X+17U+1X+1U+7X+7U+AAMU}{MX+MU+DX+DU+TX+TU+AAMU}$ (*N*-3 demethylation ratio)	8	0.14	0.22	0.04	0.47

Ratios calculated from 24-hour collection of urine.
CL/W, Caffeine clearance; CL/LWT, caffeine clearance expressed as a function of lean body mass (L/hr/kg); *p*, level of significance for regression ($p = 0.05$ is significant); r^2, coefficient of determination adjusted for degrees of freedom.
*Best ratio identified by stepwise multiple regression to explain the variation in caffeine clearance (CL/W).

Statistical analysis. Hypothesis testing used repeated-measures ANOVA, and the Tukey post-test was used for multiple comparisons when there were three or more conditions. When two conditions were compared, either a two-tailed *t* test or a nonparametric Mann-Whitney test was applied. Linear and stepwise multiple regression was used for the correlations and the coefficient of correlation (or r^2) was corrected for the degrees of freedom.

Caffeine metabolite ratios. The ratios that are displayed in the results section of this article include ratios that have been described in the literature to reflect CYP1A2 activity. The metabolism of caffeine and the various relationships among the caffeine metabolites that have been explored in this study are described in Fig. 1. The ratios used include AAMU+1X+1U/17U, AAMU+1X+1U/17X,[2,16] paraxanthine/caffeine test (17X+17U/137X),[20] and a series of suggested demethylation ratios.[4] Other ratios of caffeine metabolites that have been reported to correlate with the activity of enzymes other than CYP1A2 are included in the analysis. These other ratios include AAMU/AAMU+1X+1U, the acetylation ratio of caffeine; the 1U/1X ratio, which is thought to reflect xanthine oxidase activity; and the 17U/17X ratio, which has been postulated to reflect the activity of enzymes involved in hydroxylation of 17X.[6,16] The use of these non-CYP1A2 or "control" ratios has a number of purposes: (1) to observe the effect of liver disease on their values, (2) to help validate the significant correlations observed between some ratios and caffeine clearance and so to demonstrate that these relationships were not random events, and (3) to see whether a combination of ratios that describe the activity of different enzymes

Table IV. Correlation of caffeine clearance with caffeine metabolite ratios in control subjects plus subjects with cirrhosis: experiment 1

No.	Ratio	n	CL/W (L/hr/kg)		CL/LWT (L/hr/kg)	
			p	r^2	p	r^2
1	$\frac{AAMU+1X+1U}{17X}$ (paraxanthine demethylation ratio)	20	0.13	0.07	0.05	0.16
2	$\frac{AAMU+1X+1U}{17U}$ * (paraxanthine demethylation ratio)	20	<0.01	0.79	<0.01	0.77
3	$\frac{17X+17U}{137X}$ (*N*-3 demethylation ratio)	19	0.01	0.28	<0.01	0.35
4	$\frac{17U}{17X}$ (paraxanthine hydroxylation ratio)	20	0.27	0.02	0.48	0
5	$\frac{AAMU}{AAMU+1X+1U}$ (acetylation ratio)	20	0.71	0	0.90	0
6	$\frac{IU}{1X}$ (xanthine oxidase ratio)	20	0.83	0	0.61	0
7	$\frac{(DX+DU)+2(MX+MU+AAMU)}{3(MX+MU+DX+DU+TX+TU+AAMU)}$ (total demethylation ratio)	20	<0.01	0.43	<0.01	0.51
8	$\frac{17X+17U+1X+1U+7X+7U+AAMU}{MX+MU+DX+DU+TX+TU+AAMU}$ (*N*-3 demethylation ratio)	20	<0.01	0.41	<0.01	0.47

*Best ratio identified by stepwise multiple regression to explain the variation in caffeine clearance (CL/W).

involved in the metabolism of caffeine would correlate better to caffeine clearance.

RESULTS

Experiment 1. Table I displays the difference in clearance and half-life between healthy control subjects and subjects with cirrhosis. Clearance was markedly reduced and half-life prolonged in subjects with cirrhosis. Table II shows the changes in CMRs that occur with liver disease. AAMU+1X+1U/17U (ratio 2) was significantly decreased in subjects with cirrhosis and showed the greatest difference between control subjects and subjects with cirrhosis. Although AAMU+1X+1U/17X (ratio 1) is also decreased, the large intersubject variation precluded any statistical difference. The demethylation ratios suggested by Carrier et al.[4] had particularly low coefficients of variation. Thus, significant differences were observed between control subjects and subjects with cirrhosis, despite relatively small mean differences. When CMRs were correlated with caffeine clearance in subjects with cirrhosis, significant relationships were observed for AAMU+1X+1U/17U and, to some extent, the demethylation ratios (Table III and Fig. 2). The correlation improved slightly when caffeine clearance was expressed as a function of lean body mass. Similar findings were observed for the control group alone[5] and when both groups were combined (Table IV and Fig. 3). Stepwise multiple regression did not reveal any combination of ratios to be superior to AAMU+1X+1U/17U in the correlation to caffeine clearance.

A significant relationship between AAMU+1X+1U/17U and caffeine clearance was not observed with the spot urine tests at all times of day in the subjects with cirrhosis, probably because the results from some subjects had to be rejected because concentrations of major caffeine metabolites in their spot urine samples were too low to measure accurately.

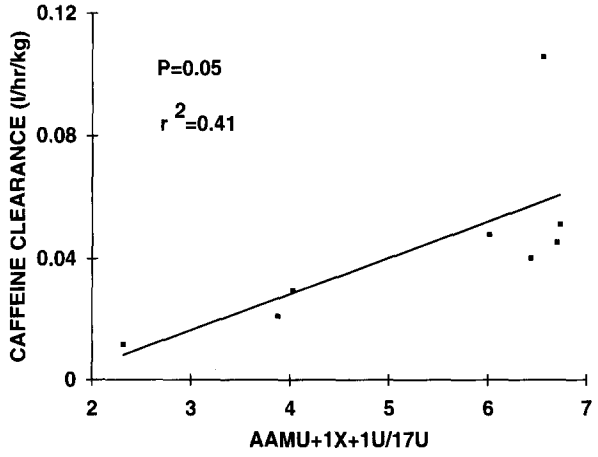

Fig. 2. Relationship between AAMU+1X+1U/17U and caffeine clearance in subjects with cirrhosis (regression analysis): experiment 1. *Solid line* indicates the regression line.

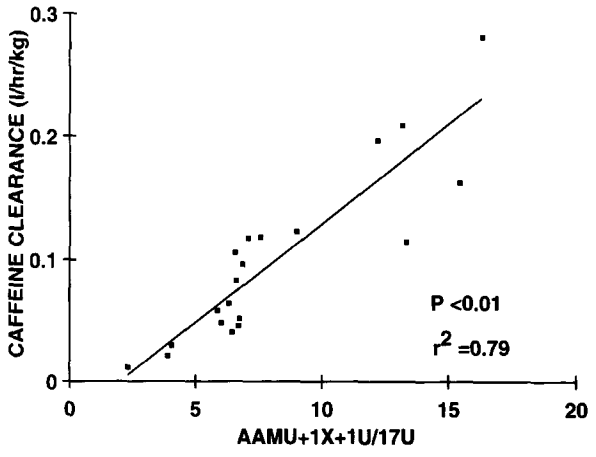

Fig. 3. Relationship between AAMU+1X+1U/17U and caffeine clearance in all subjects (control subjects and subjects with cirrhosis combined): experiment 1. *Solid line* indicates the regression line.

This lessened the power of finding significant correlations. The spot urine samples collected in the afternoon had the most subjects with cirrhosis who could be evaluated (seven subjects) and the afternoon samples most closely correlated with caffeine clearance (p = 0.01; r^2=0.69).

In subjects with cirrhosis, prothrombin time was closely correlated to both caffeine clearance and AAMU+1X+1U/17U, but GEC was correlated only to caffeine clearance (Fig. 4). When the control group was added, a relationship could be observed for AAMU+1X+1U/17U and GEC (p = 0.006; r^2 = 0.31). No relationship was observed between caffeine clearance or AAMU+1X+1U/17U and Child-Pugh score or serum albumin concentration. A weak correlation was observed between serum bilirubin and caffeine clearance (p = 0.07; r^2 = 0.38). GEC correlated weakly with prothrombin time (p = 0.037; r^2 = 0.33), serum albumin (p = 0.02; r^2 = 0.42), and serum bilirubin concentrations (p = 0.06; r^2 = 0.26), but not with the Child-Pugh score.

Experiment 2. In experiment 2, a number of CMRs were calculated from spot urine collections in control subjects and subjects with chronic hepatitis and cirrhosis. Table V shows that the AAMU+1X+1U/17U ratio was significantly different between control subjects and all groups with cirrhosis. There was no difference between control subjects and subjects with chronic hepatitis. However, Fig. 5 shows a large degree of overlap between any of the groups. There was a trend for the smokers of each group to have higher values than nonsmokers, but this did not reach statistical significance except for the group with chronic hepatitis. Again, no significant relationship was seen between Child-Pugh scores and AAMU+1X+1U/17U.

DISCUSSION

The effects of liver dysfunction on the relationship between CMRs and various enzymatic activities they are thought to reflect have not been studied previously. Because these particular surrogate measures are ratios, changes in both numerator and denominator may occur in hepatic dysfunction, making changes in ratios hard to predict. With AAMU+1X+1U/17U, the metabolites of caffeine in the numerator are demethylation products of paraxanthine catalyzed by CYP1A2; the metabolite 17U in the denominator results from hydroxylation of paraxanthine, which is thought to be catalyzed by CYP2A6 and in part by CYP1A2.[21] It has been speculated that the value of this ratio would not change with hepatic dysfunction because the enzymes involved might be affected equally by the hepatic dysfunction.[1] In this case, this ratio may not reflect caffeine clearance.

Our first experiment examined the relationship between various CMRs and caffeine clearance (and presumably CYP1A2 activity) in subjects with cirrhosis and in simulated "real world conditions." That is, subjects were enrolled whether they were regular consumers of caffeine or not and they were

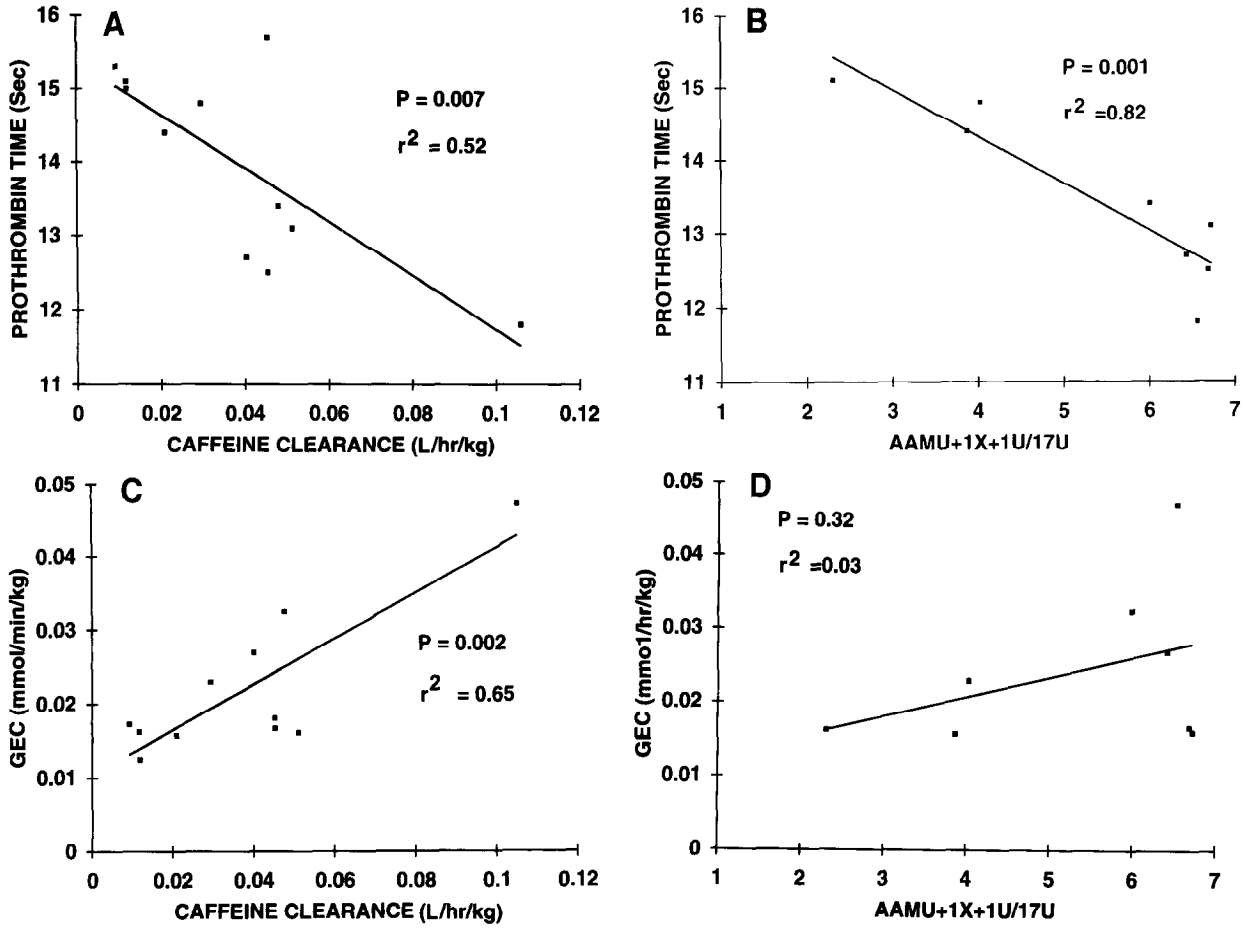

Fig. 4. Caffeine clearance and AAMU+1X+1U/17U relationship to prothrombin time (**A** and **B**) and galactose elimination capacity (GEC) (**C** and **D**) in subjects with cirrhosis: experiment 1. *Solid lines* indicate regression lines.

allowed to continue their usual habit while they were being studied. For those who did not normally consume caffeine, a single dose of caffeine was given. The use of stable isotope-labeled caffeine allowed us to examine the relationship between CMRs and caffeine clearance no matter what the oral caffeine dosing regimen was. We have previously shown that AAMU+1X+1U/17U is correlated to caffeine clearance in the setting of both single and multiple doses of oral caffeine as long as the dose is not too large.[5]

The second experiment was designed with the possibility in mind that CMRs might be used in future clinical practice. Thus the CMRs were measured from spot urine samples as subjects attended a hepatology clinic. Their caffeine consumption in the 24 hours before clinic attendance was quite variable. Unfortunately, the problem of designing experiments in which subjects consumed variable doses of caffeine was that some subjects had concentrations of one of the major metabolites of caffeine below the level at which we could accurately quantitate with confidence, and this lessened the power of the two experiments presented.

The subjects with cirrhosis in experiment 1 had severe impairment in their ability to eliminate caffeine (Table I). The average half-life in the group of subjects with cirrhosis was 21 hours. These findings are consistent with those reported by other researchers,[8,10,13,22] although our study is the first study of which we are aware that has characterized caffeine disposition in patients with cirrhosis using a blood sampling paradigm that was greater than three half-life values in duration (the optimal duration for sampling when characterizing pharmacokinetics). Galactose was given immediately after caf-

Table V. Caffeine metabolite ratios calculated from spot urine samples in control subjects and subjects with liver disease: experiment 2

Ratio	*Control (n = 17)*	*Chronic hepatitis (n = 8)*	*All cirrhosis (n = 27)*	*Child's A (n = 13)*	*Child's B/C (n = 14)*
$\frac{AAMU+1X+1U}{17X}$ (paraxanthine demethylation ratio)	6.35	6.11	6.18	6.36	6.03
$\frac{AAMU+1X+1U}{17U}$ (paraxanthine demethylation ratio)	6.15	6.89	4.25*	4.17*	4.85*
$\frac{17X+17U}{137X}$ (*N*-3 demethylation ratio)	8.25	11.26	5.74*	5.20	6.02
$\frac{AAMU}{AAMU+1X+1U}$ (acetylation ratio)	0.23	0.36	0.34	0.45	0.26
$\frac{1U}{1X}$ (xanthine oxidase ratio)	1.36	1.61	1.74	1.68	1.82
$\frac{17U}{17X}$ (paraxanthine hydroxylation ratio)	0.82	0.92	1.57	1.57	1.59
$\frac{(DX+DU)+2(MX+MU+AAMU)}{3(MX+MU+DX+DU+TX+TU+AAMU)}$ (total demethylation ratio)	0.56	0.58	0.54	0.54	0.54

All values expressed as median.
*Significant difference from control group ($p < 0.05$).

feine injection. A previous study has shown that the combination has no effect on the measurement of caffeine clearance or GEC.[23] GEC was measured because it is thought to be a measure of functioning liver mass[24] and is independent of CYP1A2 activity.

A number of CMRs were affected by cirrhosis (Tables II and V) and, in particular, the decline in AAMU+1X+1U/17U was correlated with the decline in caffeine clearance observed in this group (Tables III and IV). AAMU+1X+1U/17U was the only ratio for which there was a consistent significant difference between the groups with cirrhosis and the control group, although others may have been so if we could have measured all the caffeine metabolites in all the subjects that were enrolled in the study. The change in this ratio suggests that demethylation and CYP1A2 activity are more severely affected by cirrhosis than the hydroxylation of paraxanthine to 17U. This is a surprising result because it suggests that there is differential preservation of P450 enzymes in cirrhosis; that is, if the in vitro studies are correct,[21] then the activity of CYP2A6 is more preserved than the activity of CYP1A2 in severe liver disease. This would be evidence to suggest that cirrhosis is characterized by a number of "sick" hepatocytes rather than just a decreased mass of normal functioning cells. Of course, other explanations for this observation may also exist, such as differences in liver blood flow or renal excretion of caffeine metabolites in subjects with cirrhosis compared with healthy subjects.

As a surrogate measure of caffeine clearance, hepatic function, or CYP1A2 activity, AAMU+1X+1U/17U is not as sensitive as the direct measurement of caffeine clearance. In Table I, caffeine clearance in subjects with cirrhosis decreased to 28% of the control's value (31% if the eight subjects with cirrhosis were used, where caffeine clearance and AAMU+1X+1U/17U could both be measured), whereas AAMU+1X+1U/17U decreased only to 53% (mean values, Table II). The change in the value of AAMU+1X+1U/17U was attenuated presumably because the production of 17U depends, in part, on the activity of CYP1A2.

The demethylation ratios, while showing a significant difference between subjects with cirrhosis and control subjects and showing a significant correlation to caffeine clearance, have previously been shown to have a variable correlation to caffeine clearance in the control groups.[5] This may be due to the effect of urine flow rates on the renal clearance of caffeine and dimethylxanthines.[25,26] The coefficients of variation for the demethylation ratios are much smaller than the known variation observed in the in vitro assessment of CYP1A2 activity[6] and observed in caffeine clearance in this study (Table I) and many other studies.[27] Finally, the value of the total demethylation ratio changed only by 10% when the group of subjects with cirrhosis was compared with the control subjects (mean values, Table II), a change that is disproportionately small compared with the change in caffeine clearance.

The AAMU+1X+1U/17X ratio, which has been used by some investigators, was also decreased in subjects with cirrhosis (see results in Table III and Table V). However, the large variation in its value, perhaps due to changes in renal clearance of paraxanthine (17X) with changes in urine flow, precluded any significant relationship with caffeine clearance. This has been previously observed in healthy subjects.[5] The paraxanthine/caffeine ratio (17X+17U/137X) was also decreased in the group of subjects with cirrhosis and was correlated with caffeine clearance, although not as highly correlated as AAMU+1X+1U/17U. Of note, the calculation of this ratio was not carried out following the strict protocol as it was originally described.[20] No significant differences were seen between control subjects and subjects with cirrhosis with 1U/1X, 17U/17X, or the acetylation ratio AAMU/AAMU+1X+1U.

In control subjects and in subjects with cirrhosis, AAMU+1X+1U/17U is consistently correlated to caffeine clearance. With spot urine collections, the correlation was variable in the subjects with cirrhosis, but we believe this was a function of the small number of subjects available to be analyzed. We have previously shown that AAMU+1X+1U/17U was consistently correlated to caffeine clearance in healthy subjects, even with spot urine collections.[5] These data support the use of AAMU+1X+1U/17U as a measure of CYP1A2 activity in subjects with cirrhosis when it is calculated from an afternoon spot urine sample; the subjects had consumed most of their caffeine in the morning in either single or multiple doses. Because AAMU+1X+1U/17U is highly correlated with caffeine clearance, the urine ratio could be used instead of caffeine clearance in assessing hepatic function, particularly if sequential measurements are required. We have shown previously that the intraindividual variation in this ratio is much less than the interindividual variation.[5]

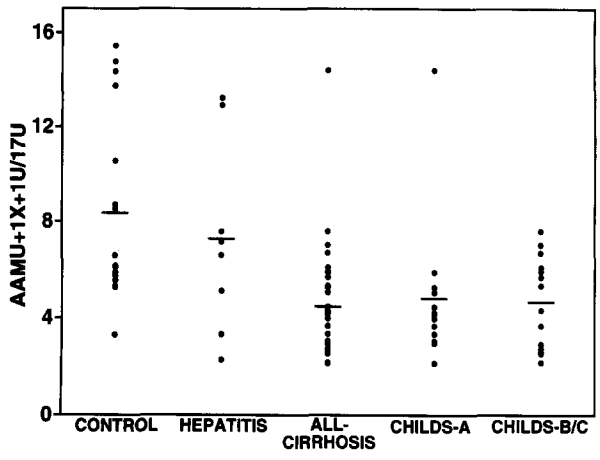

Fig. 5. AAMU+1X+1U/17U calculated from single spot urine samples in control subjects and in subjects with liver disease: experiment 2. *Horizontal bars* indicate mean values.

The proposal that AAMU+1X+1U/17U could be used as a quantitative measure of hepatic dysfunction is supported by the fact that AAMU+1X+1U/17U had a significant correlation similar to that of caffeine clearance to prothrombin time (Fig. 4). Although the ratio did not show a significant relationship to GEC in the subjects with cirrhosis, as caffeine clearance did, there were only eight subjects who could be used for the correlation between AAMU+1X+1U/17U and GEC, whereas there were 11 subjects used for the correlation between caffeine clearance and GEC. When the control group was added, both caffeine clearance and AAMU+1X+1U/17U were significantly correlated to GEC. A negative finding was the lack of correlation for both caffeine clearance and AAMU+1X+1U/17U with the Child-Pugh score. Although two previous studies showed a relationship between caffeine clearance and the Child-Pugh score,[12,13] our study may have had an insufficient number of subjects with cirrhosis and, more importantly, enrolled subjects with little variation in regard to the Child's classification.

Although the average value for AAMU+1X+1U/17U can distinguish cirrhosis from healthy subjects, it cannot be used as a diagnostic test for an individual patient because of the large degree of overlap in values observed (Fig. 5). No difference was seen

between control subjects and subjects with chronic hepatitis. This would be expected in view of previous reports that caffeine clearance is only marginally different between the two groups.[10,11]

In conclusion, the paraxanthine demethylation ratio (AAMU+1X+1U/17U) was significantly decreased in subjects with cirrhosis. Because this ratio reflects relative drug metabolism by two competing pathways, the change suggests differential preservation of P450 enzymes in patients with cirrhosis. AAMU+1X+1U/17U correlated with caffeine clearance and prothrombin time. AAMU+1X+1U/17U calculated from a spot afternoon urine sample has the potential to estimate CYP1A2 activity and might be useful as a simplified measure of caffeine clearance in subjects with cirrhosis. On the other hand, the magnitude of change in the value of AAMU+1X+1U/17U when comparing patients with cirrhosis and healthy subjects was much less than the change in caffeine clearance, the latter presumably the best in vivo marker of CYP1A2 activity. For quantitative studies of CYP1A2 activity and hepatic function, caffeine clearance is the preferred surrogate measure.

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