Validity of the ^{13}C-Caffeine Breath Test as a Noninvasive, Quantitative Test of Liver Function

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The properties of caffeine render it an ideal substrate for a quantitative test of liver function. The aim of this study was to determine whether the caffeine breath test (CBT) using orally administered ^{13}C-caffeine correlates reliably with plasma caffeine clearance and reflects varying degrees of liver dysfunction. The CBT was performed in 25 healthy controls; 20 subjects with noncirrhotic, chronic hepatitis B or C; and 20 subjects with cirrhosis. Plasma caffeine clearance was assayed simultaneously with the CBT in a cohort of these subjects. Over a broad range of caffeine clearances, the CBT exhibited a highly significant correlation with plasma clearance ($r = 0.85$, $P < .001$). Cirrhotic patients were characterized by significantly reduced CBT values (1.15 ± 0.75 △‰ mg^{-1}) compared with controls (2.23 ± 0.76; $P = .001$) and hepatitic patients (1.83 ± 1.05; $P = .04$). There was a significant inverse relationship between the CBT and Child-Pugh score ($r = -.74$, $P = .002$). The intraclass correlation coefficient between repeated CBTs in 20 subjects with normal and cirrhotic livers was 0.89. Although smoking was associated with an 86% to 141% increase in CBT in all groups, the CBT was able to distinguish control, hepatitic, and cirrhotic smokers (5.36 ± 0.82, 3.63 ± 1.21, and 2.14 ± 1.14, respectively, $P = .001$). Multivariate analysis revealed that only smoking ($P < .001$) and disease state ($P = .001$) were significant predictors of the CBT. In conclusion, the ^{13}C-CBT represents a valid indicator of plasma caffeine clearance and correlates reproducibly with hepatic dysfunction. (HEPATOLOGY 2003;38:1227-1236.)

There is an unfulfilled clinical need for a quantitative test of liver function that is safe, convenient, and yields reliable data on disease severity and prognosis. Caffeine is a ubiquitous substance, the pharmacokinetics of which have been well elucidated in normal humans.[1,2] It has high oral bioavailability and undergoes almost exclusive hepatic metabolism, principally via demethylation by cytochrome P450 1A2 (CYP1A2) to CO_2.[3] It has a low extraction ratio and low plasma binding,[4] and at the test doses used may be considered innocuous. These characteristics render caffeine an ideal substrate for a breath test of hepatic function.

Since the initial observation of a prolonged caffeine half-life in a patient with alcohol-induced liver disease,[5] plasma clearance studies have shown impaired elimination of caffeine in subjects with cirrhosis.[4] Serum and salivary caffeine clearance have been shown to be predictive of mortality in cirrhosis[6,7] and salivary caffeine clearance correlates with the more traditional quantitative assays, indocyanine green clearance, galactose elimination capacity, and the aminopyrine breath test.[8] However, significant technical limitations exist in evaluating caffeine kinetics in blood and saliva. Measurement of plasma caffeine clearance involves repeated blood sampling over an extended period of time. Because of cross-reactivity with other methylxanthines,[9] the salivary caffeine clearance test may not be wholly specific for caffeine and, furthermore, variability of caffeine concentrations may be induced by factors such as salivary pH level and flow rate.[10]

After investigations in rats, the feasibility of a caffeine breath test (CBT) in normal humans was examined.[11] To date, the major role of the CBT has been to study the effect of various drugs on CYP1A2 activity.[12-15] There has been only one study in which the CBT has been evaluated in subjects with liver disease.[16] After the intravenous administration of radioactively labeled ^{14}C-caffeine, the

Abbreviations: CBT, caffeine breath test; CYP1A2, cytochrome P450 1A2.

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breath test was shown to correlate with plasma caffeine clearance and indicate varying degrees of liver functional impairment. These results, which did not include the effect of cigarette smoking on CYP1A2 activity, have not been validated and no prospective studies comparing the CBT with traditional markers of disease severity and prognosis have been performed. Further, the intravenous route is invasive and repeated exposure to a radioactive substance, if the test was applied serially to monitor disease evolution or therapeutic response, is undesirable. Studies on the correlation between the ^{13}C-CBT and plasma caffeine clearance and the reproducibility of the CBT in subjects with liver disease are lacking.

In view of the apparent suitability of caffeine as a substrate of liver function and the limitations of previous methods of its measurement, the current study was designed to determine if the CBT, using the stable isotopic label ^{13}C administered orally, represents a valid indicator of plasma caffeine clearance in subjects with and without liver disease. A novel, simplified, breath test parameter differentiating the liver diseases was established. The influence of smoking on caffeine metabolism among subjects with hepatic dysfunction was evaluated and the intrasubject reproducibility of the CBT also was assessed.

Patients and Methods

Participants. Twenty ambulatory patients with untreated, noncirrhotic, chronic hepatitis B or C, and 20 subjects with cirrhosis were recruited from outpatient clinics and specialist rooms. None of the patients had known significant pulmonary disease and all but one cirrhotic patient had normal serum creatinine values. All subjects with liver disease had undergone liver biopsies confirming their diagnosis except 4 cirrhotic subjects whose diagnosis of cirrhosis was based on consistent clinical, laboratory, and radiologic findings. Where appropriate, liver histology was scored according to the method described by Scheuer.[17] Subjects with cirrhosis were graded according to the Child-Pugh score.[18] Twenty-five healthy volunteers were studied as controls. All had normal clinical and biochemical findings, took no regular medication, and had hepatitis B and C excluded by serology. All smokers had been smoking for at least 5 years and smoked 15 to 40 cigarettes/d. Ex-smokers had ceased smoking for at least 12 months and were considered current nonsmokers. The clinical and laboratory data of all study participants are summarized in Table 1. All subjects gave written, informed consent for the study, which was approved by the Central Sydney Area Health Services Human Research Ethics Committee and the Human Ethics Committee of the University of Sydney.

Table 1. Clinical and Laboratory Data of Study Subjects

	Control	Chronic Viral Hepatitis	Cirrhosis
n	25	20	20
Sex (M/F)	12/13	14/6	14/6
Age (y)			
Mean ± SD	40 ± 15	45 ± 11	60 ± 12*
Range	23-74	28-72	38-78
Smoker/nonsmoker	5/20	7/13	6/14
Liver tests			
Bilirubin (μmol/L)	10 ± 2	12 ± 5	31 ± 42*
GGT (IU/L)	22 ± 14	50 ± 36	188 ± 250†
ALT (IU/L)	20 ± 8	106 ± 90‡	45 ± 31
AST/ALT	1.1 ± 0.4	0.6 ± 0.2‡	1.2 ± 0.5
Albumin (g/L)	44 ± 2	42 ± 2	38 ± 6†
INR	1.0 ± 0.1	1.0 ± 0.1	1.4 ± 0.5†
Platelets ($\times 10^9$/L)	231 ± 46	219 ± 51	125 ± 52§
Cause			
HBV		8	3
HCV		12	1
Alcohol			11
NASH			1
PBC			1
AIH			1
Unknown			2
Scheuer score			
Portal activity (0-4)		1.7 ± 0.7	
Lobular activity (0-4)		1.6 ± 0.6	
Fibrosis (0-4)		1.4 ± 0.6	
Child-Pugh grade			
A			12
B			5
C			3

Abbreviations: GGT, γ-glutamyltranspeptidase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; INR, international normalized ratio; HBV, hepatitis B virus; NASH, nonalcoholic steatohepatitis; PBC, primary biliary cirrhosis; AIH, autoimmune hepatitis.

*$P < .05$ vs. control and hepatitis groups.

†$P < .01$ vs. control and hepatitis groups.

‡$P < .01$ vs. control and cirrhosis groups.

§$P < .001$ vs. control and hepatitis groups.

Experimental Protocol. To ascertain the correlation between the systemic clearance of caffeine and the CBT, the first 9 controls, 7 with viral hepatitis and 14 with cirrhosis, underwent simultaneous measurement of plasma clearance and the CBT. The liver biopsies of the hepatitic patients had been performed within 6 weeks of the current studies.

Subjects abstained from caffeine-containing products and limited alcohol consumption to one standard drink (10 g ethanol) for 24 hours before testing. All nonessential medications were withheld for 48 hours before testing. After an overnight fast, subjects ingested 2 mg/kg of [3-methyl-^{13}C] caffeine (99% ^{13}C), obtained in powder form from Cambridge Isotope Laboratories (Cambridge, MA) and dissolved in 20 mL of sterile water, followed by a 40 mL water wash of the container. The quantity of caffeine consumed was approximately equivalent to 2

cups of coffee. Subjects sat quietly for 15 minutes before and throughout the CBT because physical activity influences endogenous CO_2 production and may affect CBT results.[19]

Paired breath samples were collected during normal expiration into test tubes via a straw. Venous samples were drawn via an indwelling cannula at 0, 15, 30, 45, 60, 75, 90, 105, 120, 180, 240, 360, and 420 minutes after caffeine ingestion into heparinized tubes and immediately centrifuged (3,000 rpm, 10 min) and stored at −20°C until analysis. Between the 180- and 240-minute time points, subjects were given a light, caffeine-free lunch. With the baseline venous sample, blood also was collected for hematologic and biochemical analysis performed in the central hospital laboratory according to standard methods. Subjects were observed for signs of caffeine toxicity with detailed symptom assessment, while blood pressure and pulse were monitored every hour during testing.

Once a significant correlation between breath and plasma caffeine kinetics had been established, the remaining subjects in each group underwent a 1-hour CBT, omitting collection of plasma caffeine concentrations. In the 13 remaining subjects with viral hepatitis, the 1-hour CBT was conducted on the day of their liver biopsy.

To investigate the reproducibility of the CBT, tests were conducted twice within a 1-3 week period in 20 subjects (11 control patients, 9 cirrhotic patients) under stable physiologic and environmental conditions. Tests in controls were repeated after approximately 1 week and in cirrhotic subjects after 3 weeks because of their prolonged caffeine half-life.

Sample Analysis. The ^{13}C-enrichment of expired CO_2 was determined by continuous-flow isotope ratio mass spectrometry[20] using an automated 13-Carbon Breath Analyzer (PDZ Europa, Cheshire, England). ^{13}C-enrichment was expressed as △‰, by subtracting the average predose enrichment from each postdose measurement, with respect to the international $(^{13}C/^{12}C)_{PDB}$ standard, originating from the Pee-Dee-Belemnite, a fossil limestone of the Pee-Dee-Formation in South Carolina, with a $^{13}C/^{12}C$ isotope ratio of 0.0112372.[21] The cumulative ^{13}C-enrichment over a particular time interval was calculated by averaging the measured enrichments over that time interval. For example, the cumulative 1-hour value represented the average ^{13}C-enrichment of 4 breath samples taken every 15 minutes during the first hour after administration of ^{13}C-caffeine whereas the single 1-hour CBT value represented the ^{13}C-enrichment measured 1-hour after substrate dosing. This then was expressed as a percentage of the original caffeine dose (in mg), giving rise to the composite unit for CBT results as △‰ mg^{-1}. Given that the doses of labeled caffeine were administered according to body weight, the ^{13}C-enrichments were expressed without normalization of body weight. Plasma levels of ^{13}C-caffeine were measured by gas chromatography mass spectrometry with selected ion monitoring (Model QP5000; Shimadzu, Kyoto, Japan). The coefficient of variation for caffeine concentrations up to 100 μmol/L was 7%.

Pharmacokinetic Analysis. Plasma concentration-time data of ^{13}C-caffeine were analyzed using a standard pharmacokinetic approach. The area under the concentration-time curve to the last detectable observation ($AUC_{0\text{-}t}$) was calculated using the linear trapezoidal rule. The area under the concentration-time curve from 0 to infinity ($AUC_{0\text{-}\infty}$) was calculated using the following equation:

$$AUC_{0\text{-}\infty} = AUC_{0\text{-}t} + C_t/k$$

where C_t/k is the extrapolated portion of the $AUC_{0\text{-}\infty}$, C_t is the last detected ^{13}C-caffeine concentration, and k is the elimination rate constant calculated as the absolute value of the slope of the terminal portion of the log-linear concentration-time curve. Caffeine clearance (CL) was estimated by the following formula:

$$CL/F = D/AUC_{0\text{-}\infty}$$

where F is the oral bioavailability (assumed to be 1) and D is the dose administered. The apparent volume of distribution (V/F) was calculated as follows:

$$V/F = CL/k$$

and the half-life ($t_{1/2}$) of elimination of caffeine was calculated by the equation:

$$t_{1/2} = \ln 2/k$$

where ln2 is the natural logarithm of 2.

Statistical Analysis. Results are given as mean ± SD. Means between groups were compared using one-way analysis of variance followed by *post hoc* multiple comparison procedures. Pearson's correlation coefficient was used to study the association between quantitative variables. Intraclass correlation coefficient was computed to determine the intrasubject reproducibility of the CBT. Multivariate analysis using linear regression assessed the effects of age, sex, liver disease, and smoking status on CBT parameters and tested for interactions between these covariates and the group effect. A P value of less than .05 was considered significant. Statistical analyses were performed using the statistical software package SPSS version 10.0 (SPSS Inc., Chicago, IL).

Table 2. Pharmacokinetics of Caffeine and 1-Hour CBT Results in 30 Subjects

Subject	Sex	Age (y)	Weight (kg)	Smoker	CL/F* (mL/min/kg)	V/F† (L/kg)	$t_{1/2}$‡ (h)	CBT (Δ‰ mg^{-1})
Controls								
1	M	26	80	No	2.19	0.90	4.75	2.37
2	M	27	67	No	0.99	0.94	10.90	0.91
3	M	27	71	No	1.77	0.88	5.75	2.23
4	F	25	56	Yes	4.68	1.06	2.63	4.50
5	M	44	92	No	2.46	0.74	3.46	2.23
6	M	25	77	No	1.72	0.91	6.14	1.26
7	F	24	54	No	3.12	0.76	2.82	4.28
8	M	49	65	No	2.32	0.92	4.60	2.72
9	F	43	67	No	2.41	0.76	3.66	1.91
Mean		32	70		2.41	0.87	4.97	2.49
SD		10	12		1.04	0.10	2.50	1.21
Chronic viral hepatitis								
1	M	41	65	No	2.24	0.42	2.19	1.92
2	M	54	60	No	1.12	0.57	5.92	1.67
3	F	49	55	No	0.58	0.53	10.60	0.60
4	M	31	74	Yes	4.74	1.52	3.71	3.88
5	M	72	64	No	1.22	0.71	6.72	1.37
6	M	36	91	Yes	3.75	1.13	3.48	4.05
7	M	47	68	Yes	4.35	1.04	2.76	5.32
Mean		49	68		2.57	0.85	5.05	2.69
SD		14	12		1.70	0.40	2.94	1.73
Cirrhosis								
1	F	71	75	No	1.02	1.10	12.52	1.14
2	M	76	78	No	2.14	1.06	5.72	1.24
3	F	64	67	Yes	1.82	1.01	6.42	1.44
4	M	51	63	Yes	3.30	0.65	2.29	3.79
5	F	44	56	Yes	2.44	1.21	5.75	2.89
6	M	72	76	No	0.96	0.73	8.82	0.71
7	M	78	78	Yes	5.33	1.51	3.26	2.50
8	F	56	93	Yes	1.59	0.77	5.61	1.79
9	F	42	48	No	0.67	0.59	10.22	1.84
10	M	38	101	Yes	0.20	0.76	43.93	0.45
11	M	54	63	No	0.48	1.09	26.26	0.31
12	M	61	70	No	0.21	1.01	55.81	0.54
13	M	47	65	No	1.74	1.36	9.03	0.83
14	M	73	63	No	0.44	0.78	20.30	1.56
Mean		59	71		1.60	0.97	15.42	1.57
SD		14	14		1.41	0.28	16.15	0.99

*Apparent total plasma clearance where F denotes bioavailability, assumed to be 1.

†Apparent volume of distribution.

‡Plasma half-life.

Results

Plasma Caffeine Kinetics. The caffeine dose administered was well tolerated by all subjects and there were no appreciable changes in pulse and blood pressure during testing.

The pharmacokinetics of caffeine and CBT results observed in the 9 control, 7 hepatitic, and 14 cirrhotic patients who underwent both breath and plasma caffeine clearance studies are summarized in Table 2. Caffeine clearance was reduced in cirrhotic patients (1.60 ± 1.41 mL/min/kg) compared with control (2.41 ± 1.04) and hepatitic patients (2.57 ± 1.70). This did not reach statistical significance by analysis of variance ($P = .23$) but was powered to determine the correlation between plasma clearance and the CBT, rather than to differentiate liver disease. However, the exclusion of smokers resulted in significantly pronounced differences in caffeine clearance between the 3 groups: 2.12 ± 0.63 in control, 1.29 ± 0.69 in hepatitic, and 0.96 ± 0.67 mL/min/kg in cirrhotic patients ($P = .008$). Among nonsmokers, caffeine half-life was prolonged in the cirrhotic (18.6 ± 16.5 h) compared with control (5.3 ± 2.5; $P = .03$) and hepatitic patients (6.4 ± 3.5; $P = .08$) whereas volume of distribution was similar between all 3 groups (0.87 ± 0.10 in control, 0.85 ± 0.40 in hepatitic, 0.97 ± 0.27 L/kg in cirrhotic patients; $P = .56$). The average time to peak plasma caffeine concentration in control, hepatitic, and cirrhotic patients was 48, 32, and 58 minutes, respectively ($P = .15$).

Correlation Between Caffeine Clearance and CBT. Representative examples depicting the time course of CBT values in a control, hepatitic, cirrhotic, and smoking control subject are shown in Fig. 1. Peak ^{13}C-enrichment was generally achieved by 60 minutes after caffeine administration followed by a gradual decline.

The single 1-hour CBT values of the 30 subjects who had simultaneous breath and plasma assays were compared with their corresponding plasma caffeine clearance measurements (Fig. 2). Over a broad range of clearances, the CBT exhibited a highly significant correlation ($r = 0.85$, $P < .001$) with plasma caffeine clearance. Virtually identical results were obtained using cumulative CBT parameters at 1 hour ($r = 0.84$, $P < .001$), 2 hours ($r =$

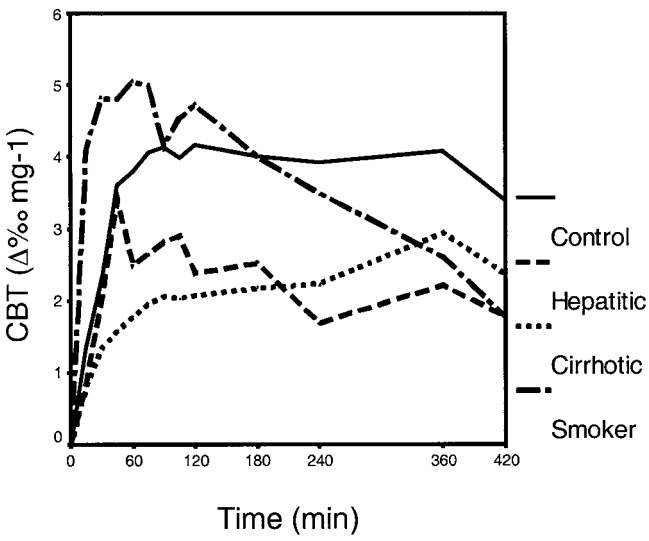

Fig. 1. Representative CBT curves for control, hepatitic, cirrhotic, and smoking control subjects.

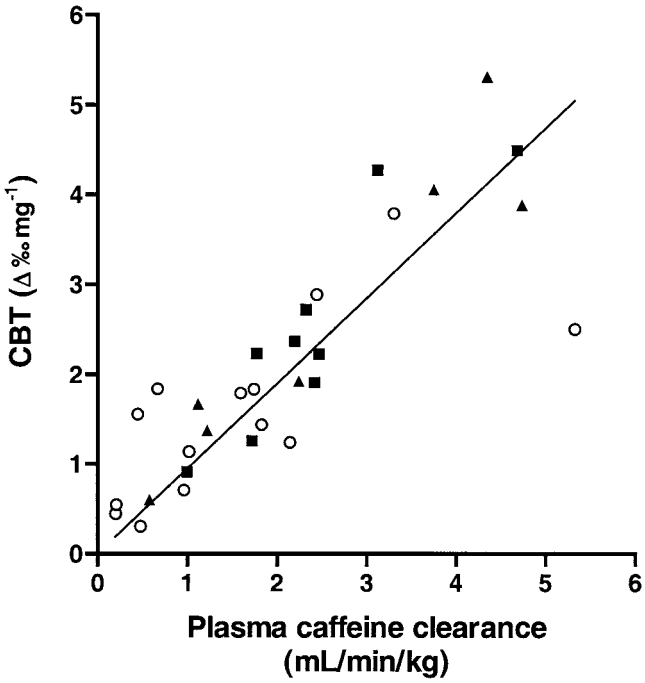

Fig. 2. Correlation between plasma caffeine clearance and 1-hour CBT in 30 subjects. The regression equation is $y = 0.79x + 0.48$. $r = 0.85$; $P < .001$. ■, Control; ▲, hepatitic; ○, cirrhotic.

0.85, $P < .001$), and 3 hours ($r = 0.85$, $P < .001$). Significant correlations also were observed with the single time-point CBT at 15 minutes ($r = 0.72$, $P < .01$), 30 minutes ($r = 0.81$, $P < .01$), and 45 minutes ($r = 0.84$, $P < .01$). The single 1-hour CBT value, however, was chosen for further analyses because it gave the best combination of correlation and reproducibility (see later).

CBT in Relation to Liver Dysfunction. Given the strong correlation between the CBT and plasma caffeine clearance, only the 1-hour CBT was performed in the remaining subjects.

Nonsmoking subjects with cirrhosis were characterized by significantly reduced CBT values (1.15 ± 0.75 △‰ mg^{-1}) compared with control (2.23 ± 0.76) and hepatitic patients (1.83 ± 1.05). *Post hoc* analysis revealed that the differences were significant between control and cirrhotic patients ($P = .001$) and between hepatitic and cirrhotic patients ($P = .04$), but not between control and hepatitic patients ($P = .20$).

Associations of the CBT with serum liver tests in nonsmoking subjects are summarized in Table 3. The CBT correlated significantly with serum albumin and platelet count, and correlated modestly with the international normalized ratio, bilirubin, γ-glutamyltranspeptidase, and aspartate/alanine aminotransferase ratio. Among cirrhotic patients, the CBT showed a significant inverse relationship with the Child-Pugh score ($r = -0.74$, $P = .002$) (Fig. 3). Among nonsmokers, the mean CBT value

Table 3. Correlation Between CBT and Serum Liver Tests in Nonsmoking Subjects

	Correlation Coefficient	*P* Value
Serum albumin	0.62	<.001
Platelet count	0.58	<.001
INR	−0.43	.006
Bilirubin	−0.39	.014
GGT	−0.39	.015
AST/ALT	−0.36	.02
AST	−0.29	.08
ALT	0.02	.91

Abbreviations: INR, International normalized ratio; GGT, γ-glutamyl transpeptidase; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

in subjects with Child-Pugh grade A cirrhosis was 1.60 ± 0.61 △‰ mg^{-1} compared with 0.84 ± 0.46 in subjects with Child-Pugh B cirrhosis and 0.28 ± 0.25 in Child-Pugh C cirrhosis ($P = .009$). Additionally, 2 of the 20 patients with cirrhosis had completely normal physical and biochemical findings; however, their cirrhotic state (proven on biopsy) was suggested by their reduced CBT values (1.14, 1.44). Given that the latter subject was also a smoker, the true CYP1A2 activity was likely to be even more depressed than the CBT result would suggest.

Reproducibility of the CBT. To investigate the reliability of the CBT, the breath test was conducted twice within 1 to 3 weeks in 20 subjects (11 controls, 9 cirrhotic patients). Tests in control patients were repeated after approximately 1 week whereas the interval in cirrhotic

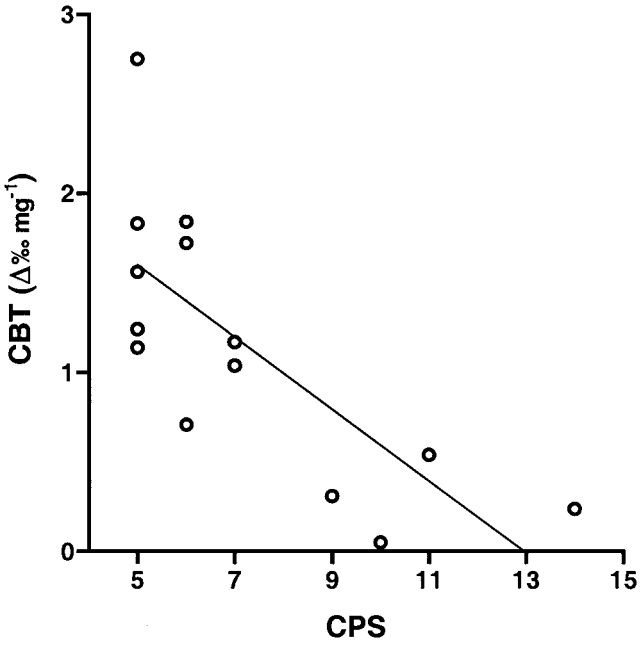

Fig. 3. Relationship between CBT and Child-Pugh score (CPS) in nonsmoking subjects with cirrhosis (n = 14). The regression equation is $y = -0.2x + 2.6$. $r = -0.74$; $P = .002$. ○, Cirrhotic.

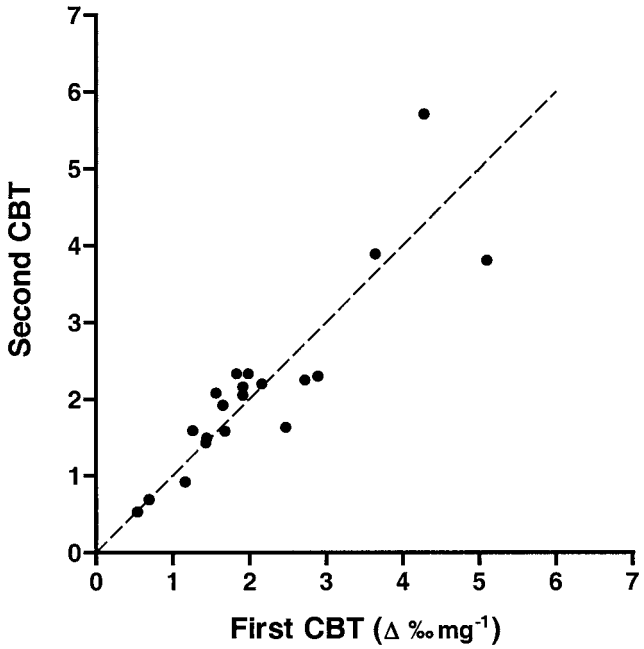

Fig. 4. Correlation between first and second CBT determinations in 20 subjects (11 controls, 9 cirrhotic patients). Identity is indicated by the dashed line. Intraclass correlation coefficient = 0.89; 95% confidence interval, 0.75-0.95.

patients was 3 weeks given their prolonged caffeine half-life. There was a highly significant correlation (intraclass correlation coefficient = 0.89; 95% confidence interval, 0.75-0.95) between repeated determinations of the 1-hour CBT (Fig. 4). Thus, the CBT showed excellent reproducibility over a broad spectrum of liver function. Intraclass correlation coefficients for repeated breath tests at 15, 30, and 45 minutes were 0.71, 0.88, and 0.82, respectively. Combining these results with their respective correlations with plasma caffeine clearance confirmed that the 1-hour CBT gave the best combination of correlation and reproducibility.

Effect of Smoking on Caffeine Metabolism. Cigarette smoking has been shown to increase caffeine clearance.[22] To test this observation, the previous cohorts of control, hepatitic, and cirrhotic subjects were analyzed in terms of their smoking status. Within all 3 groups, smokers displayed increased caffeine clearance compared with nonsmokers: 2.12 ± 0.63 versus 4.68 in 8 nonsmoking control patients versus 1 smoking control patient; 1.29 ± 0.69 versus 4.28 ± 0.50 in 4 nonsmoking versus 4 smoking hepatitic patients ($P = .001$); and 0.96 ± 0.67 versus 2.45 ± 1.74 mL/min/kg in 8 nonsmoking versus 6 smoking cirrhotic patients ($P = .046$).

The inducing effect of smoking on CYP1A2 activity also was reflected by the CBT: 2.23 ± 0.76 versus 5.36 ± 0.82 in 20 nonsmoking versus 5 smoking control patients ($P < .001$); 1.83 ± 1.05 versus 3.63 ± 1.21 in 13 nonsmoking versus 7 smoking hepatitic patients ($P = .005$); and 1.15 ± 0.75 versus 2.14 ± 1.14 Δ‰ mg^{-1} in 14 nonsmoking versus 6 smoking cirrhotic patients ($P = .035$) (Fig. 5). The increases in CBT in the control, hepatitic, and cirrhotic groups caused by smoking were 141%, 88%, and 86%, respectively. Analysis of the smoking population as a whole (n = 18) revealed significant differences in CBT values between control (5.36 ± 0.82), hepatitic (3.63 ± 1.21), and cirrhotic (2.14 ± 1.14) smokers. *Post hoc* analysis revealed that all 3 groups of smokers were significantly different from each other in terms of their CBT values ($P < .001$).

Predictive Factors for CBT. Multivariate analysis using linear regression to predict the CBT from the independent variables of age, sex, smoking status, and disease state (control, hepatitis, cirrhosis) was performed. This revealed that only smoking ($P < .001$) and disease state ($P = .001$) were significant predictors of the CBT.

Discussion

The search for a safe, reliable, and convenient quantitative test of liver function hitherto has been an elusive one. Previously proposed tests have included the aminopyrine breath test, galactose elimination capacity, indocyanine green clearance, monoethylglycinexylidide test, and antipyrine clearance. The deficiencies inherent in these tests include relative invasiveness, cumbersome performance, potential adverse effects, and complicated analysis. These factors effectively have restricted their widespread use in clinical practice. In terms of prognostication, studies have shown that these tests offer little, if

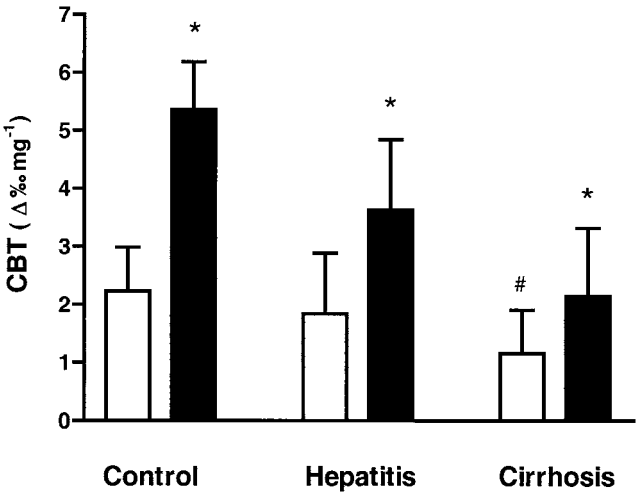

Fig. 5. Effect of smoking on CBT among control, hepatitic, and cirrhotic groups. Data are expressed as mean ± SD. *$P < .05$ versus nonsmokers within each group and versus smokers between groups; #$P < .05$ versus nonsmoking control and hepatitic groups. □, Nonsmoker; ■, smoker.

any, additional predictive information when compared with more traditional assessment such as the Child-Pugh score.[23-27]

The results from the present study support the application of the CBT as a test of quantitative liver assessment. Our data indicate that: (1) the elimination of caffeine is impaired in cirrhosis, characterized by a reduction in plasma clearance and a prolongation in caffeine half-life; (2) the oral ^{13}C-CBT using a single 1-hour measure correlates very closely with plasma caffeine clearance; (3) cirrhotic subjects show significantly reduced CBT results compared with control and hepatitic subjects; (4) CBT values are increased in smokers but still distinguish smokers of varying hepatic functional impairment; (5) the CBT appears to be a reproducible assay; and, (6) the CBT is administered easily and safely in subjects with liver disease. The CBT exhibited significant correlations with serum albumin and platelet count and correlated modestly with the international normalized ratio, bilirubin, γ-glutamyltranspeptidase, and aspartate/alanine aminotransferase ratio, markers that have been associated traditionally with hepatic dysfunction. The CBT was associated inversely with the Child-Pugh score and differentiated the grades of cirrhosis. In addition, it was able to predict the cirrhotic state in two patients with biopsy-proven cirrhosis who had completely normal physical and laboratory findings but significantly reduced CBT values.

Although these findings are in general agreement with previously published studies[1-4,16] and further extend their results, certain differences and limitations need to be considered. We elected to use the oral route of substrate delivery so that the test was truly noninvasive. This may have resulted in potential delays in absorption of caffeine, particularly in those subjects with cirrhosis. However, it has been shown that the time to peak plasma caffeine concentrations as well as peak caffeine levels after oral administration are not significantly different between cirrhotic and control patients,[4] suggesting that differences in substrate absorption do not significantly contribute to the altered pharmacokinetics of caffeine observed in cirrhosis. This was corroborated by our pharmacokinetic and breath curve data and thus it is reasonable to assume consistent and predictable high oral bioavailability of ^{13}C-caffeine in all grades of liver disease. In calculating the plasma kinetics of caffeine, we limited the collection of caffeine concentrations to 7 hours, despite an observed range of mean caffeine half-lives of 5 (control) to 15 hours (cirrhotic). Notwithstanding the logistical difficulties of a more prolonged collection time and given that the mono-exponential decline of plasma caffeine levels is maintained beyond 4 to 5 half-lives,[4] we felt that the collection period of 7 hours was adequate. Moreover, when the data were re-analyzed using the finite area under the concentration-time curve to the last detectable observation ($AUC_{0\text{-}7\ h}$) instead of that to infinity, the correlation between plasma and breath caffeine measurements still was maintained ($r = 0.74$, $P < .001$).

Among nonsmokers, CBT values in subjects with cirrhosis were approximately 50% lower than controls whereas subjects with viral hepatitis had values 20% lower than controls. The differences were statistically significant between control and cirrhotic patients and between hepatitic and cirrhotic patients, but not between control and hepatitic patients. The similarity in CBT between control and hepatitic groups is not surprising given that the hepatitic patients had only mild degrees of hepatic inflammation and fibrosis and minor derangement of liver enzyme levels (Table 1). A type II error is possible due to the relatively small samples, and performance of the CBT on larger numbers of patients with viral hepatitis may magnify the differences in caffeine breath kinetics. Alternatively, liver function in these subjects with chronic hepatitis truly may be unimpaired or caffeine metabolism is either insensitive to or unaffected by their disease process to date. Longitudinal studies during the natural progression of chronic viral hepatitis or serial estimations before and after successful antiviral treatment may partly answer this question. It also should be recognized that the cirrhotic group generally was well-compensated in terms of the severity of their liver disease (12 patients with Child-Pugh A, 5 with B, and 3 with C cirrhosis). It is likely that inclusion of subjects with more severe cirrhosis would have accentuated the differences in CBT values even further compared with subjects with lesser degrees of liver dysfunction.

A major consideration in the interpretation of the CBT as a measure of hepatic function is the influence of smoking on caffeine metabolism. Reassuringly, the CBT was able to detect the well-established inducing effect of smoking on caffeine clearance,[22] providing further support that the breath test reflects the activity of CYP1A2. Although smoking increased CBT values across all 3 groups, importantly the CBT discriminated the different degrees of liver impairment among the smokers. In fact, the differences in CBT according to liver status were more apparent in smokers than in nonsmokers, particularly when comparing control and hepatitic subjects in whom *post hoc* analysis showed a statistically significant difference that was not found among nonsmokers. The increases in CBT caused by smoking in the control group averaged 141% compared with 88% and 86% in the hepatitic and cirrhotic groups, respectively. The degree of induction of hepatic metabolizing enzymes by exogenous substrates may represent a useful marker of hepatic func-

tion. A recent study showed that the inducibility of microsomal liver function assessed by the aminopyrine breath test may indicate compromised liver reserve in cirrhosis.[28] In the present study, the less impressive increase of CBT results by smoking in the hepatitic and cirrhotic groups compared with controls may reflect impaired liver function in those subjects. Further studies exploring the effects of smoking on CYP1A2 activity including dose-response relationships and the reversibility of smoking cessation on caffeine kinetics are warranted.

Despite these limitations, it is remarkable that the correlation ($r = 0.85$) between plasma caffeine clearance and the 1-hour CBT is maintained over a wide range (0.20-5.33 mL/min/kg) of clearances (Fig. 2). Such linearity between plasma and breath measures is noteworthy considering the varying influences of the liver disorders studied and the potential confounding effects of smoking, age, and sex. Multivariate analysis confirmed that the only independent predictors of the CBT were smoking status and disease state. This is in accord with previous studies showing that caffeine metabolism does not appear to be influenced by age beyond adolescence[29,30] and sex.[30,31] It generally has been accepted that the 2-hour cumulative exhalation of labeled CO_2 represents the best parameter of caffeine clearance in the adult.[32] Our data suggest that a single 1-hour measure is equally informative and simplifies the test. Moreover, we have shown that this parameter is highly reliable in terms of its reproducibility in subjects with a normal or cirrhotic liver.

That CYP1A2 activity is of particular relevance to hepatic dysfunction is supported by the observation that cytochrome P450 proteins appear to be altered differentially in severe chronic liver disease.[33] Examination of explanted livers has revealed that CYP1A2 expression is reduced invariably and profoundly in both cholestatic and noncholestatic types of liver disease whereas the activities of other P450 proteins appear to undergo less pronounced and variable alterations, their expression being influenced by the nature of the liver injury.[33] The clinical significance of CYP1A2 is highlighted further by its central role in the activation of carcinogenic arylamines.[34] Estimation of CYP1A2 activity via the CBT may be useful in the characterization of arylamine N-oxidation phenotypes to determine if hepatic levels of CYP1A2, as affected by environmental or genetic factors, contribute to inter-individual differences in susceptibility to arylamine-induced cancers. The induction of CYP1A2 activity by direct or passive smoking may explain the carcinogenic effects of tobacco smoke (*i.e.*, through enhanced activation of carcinogens). The possible association between CYP1A2 activity and carcinogenesis is an area that deserves further evaluation.

An important consideration in the acceptance of a new test in clinical practice is cost and availability. Depending on the amount ordered, the cost of [3-methyl-^{13}C] caffeine from Cambridge Isotope Laboratories for the average subject is US $40. The analysis of breath samples is performed simply by using a ^{13}C breath analyzer (isotope ratio mass spectrometer), the same machine used for ^{13}C-urea breath testing for *Helicobacter pylori* infection. Such machines are widely available commercially and do not require specialist analytical expertise. We believe that the overall cost of the CBT is not prohibitive, especially when compared with other quantitative functional assays with their complex analysis and cumbersome performance characteristics. In certain situations, the information derived from the CBT in conjunction with other available data may obviate the need for liver biopsy or sway the clinician to accept or reject a particular intervention and this may lead to substantial savings in health care. Should future studies support the value of the test in a variety of clinical contexts and result in its greater use, the overall cost of the assay should decline.

A significant advantage of the CBT over other quantitative assays is the stability of breath samples that can be stored for later testing. Although rigorous stability testing has not yet been performed with the CBT, extrapolating the stability data on ^{13}C-urea breath samples,[35] which are essentially the same sample, would suggest that CBT samples are similarly stable. The transportability of the CBT would thus allow samples to be collected in remote areas, transferred by mail, and analyzed at centers possessing a breath analyzer.

The potential clinical and research applications of the CBT are myriad. Because the Child-Pugh score has been shown to yield prognostic information,[27,36] the close association shown between the CBT and Child-Pugh score suggests that the breath test also has prognostic potential in cirrhosis. What is also unknown is the prognostic ability of the CBT in individuals with early liver disease. To this end, longitudinal studies pre- and post-antiviral therapy comparing the breath test with histology in patients with noncirrhotic, chronic viral hepatitis are underway. Early data on patients with nonalcohol-induced steatohepatitis suggest a surprisingly low level of CYP1A2 activity in such patients. The associations of the CBT with insulin resistance, oxidative stress, and the degree of hepatic steatosis and fibrosis in patients with nonalcohol-induced steatohepatitis would be of tremendous interest given the emerging incidence of obesity and recognition of the clinical sequelae of fatty liver disease. Other applications of the CBT include its role in acute liver failure, transplant hepatology, estimating hepatic functional reserve to determine the safety of hepatic resection, study of

hepatic disorders of pregnancy and pediatrics (stable isotope), prediction of drug hepatotoxicity and tailoring of pharmacotherapy (*e.g.*, chemotherapy), and examining the effects of aging on the liver. An intriguing research focus already alluded to is the connection of CYP1A2 activity and carcinogenesis.

In conclusion, the present study confirms that the oral ^{13}C-caffeine breath test represents a valid indicator of plasma caffeine clearance and correlates reliably with hepatic dysfunction. The safety and sensitivity of caffeine in the evaluation of hepatic metabolism and the ease of nonradioactive isotopic breath analysis after simple oral administration make the CBT an appealing choice for quantifying liver function. We propose to advance the CBT as another way of measuring one of the many liver functions currently assessable. Just as serum albumin and the international normalized ratio reflect hepatic synthetic function and bilirubin estimates biliary excretory function, the CBT provides a convenient assay of microsomal P450 metabolic activity. It thus may be viewed as a complementary test in the overall assessment of liver status to be taken in context with laboratory, radiologic, and histologic data where available. More provocative perhaps is the notion that histology may not be the most important determinant of prognosis in various liver diseases, indicating as it does only morphologic changes associated with diseased liver without any information about the essential metabolic processes that underline the liver's role in sustaining life. Quantitative estimation of these inherent yet elusive processes may represent the most significant predictor of clinical outcomes.

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