Dose-dependency of caffeine metabolism with repeated dosing

Some recent epidemiologic studies have reported a nonlinear dose-response in the relationship between coffee consumption and health risks, such that the risks increase disproportionately to the increase in dose. Assuming caffeine contributes to the adverse health effects of coffee, a possible explanation for the nonlinear dose-response relationship is dose-dependent metabolism of caffeine. We examined the hypothesis that under chronic dosing conditions the metabolism of caffeine is dose-dependent. Nine healthy subjects were given, in randomized 5-day treatment blocks, placebo, 4.2 (low) and 12 (high) mg/kg/day caffeine in decaffeinated coffee, in six divided doses spaced throughout the day. On the third day of each dosing period, 25 mg of stable-isotope labeled caffeine (2-^{13}C, 1,3-$^{15}N_2$) was given intravenously. Clearance of labeled caffeine fell from 0.118 (placebo treatment) to 0.069 (low dose; $p < 0.005$) and to 0.054 (high dose; $p < 0.001$) L/hr/kg. The formation and metabolite clearances of paraxanthine, the major primary metabolite of caffeine, also decreased comparing the low and high doses ($p < 0.05$). We conclude that caffeine metabolism is dose-dependent, resulting in nonlinear accumulation of methylxanthines in the body. Dose-dependent metabolism of caffeine may explain in part why people who drink large amounts of coffee are at greater risk for cardiovascular disease. (CLIN PHARMACOL THER 1990;48:277-85.)

Charles P. Denaro, FRACP, Christopher R. Brown, MD, Margaret Wilson, MS, Peyton Jacob III, PhD, and Neal L. Benowitz, MD *San Francisco, Calif.*

Caffeine (1,3,7-trimethylxanthine) is the primary psychoactive and cardioactive component of coffee and tea, and its human pharmacology, particularly with repeated doses as is relevant to the usual pattern of use, has been incompletely characterized. Interest in the health consequences of chronic caffeine consumption has been stimulated by several recent epidemiologic studies suggesting an association between coffee consumption and increased serum cholesterol concentrations, an increased risk of ischemic heart disease, and decreased birth weight of the newborn.[1-7] Most of these studies have found a dose-related increase in particular health risks with increasing levels of coffee consumption. It is noteworthy that some studies have reported a nonlinear dose-response relationship between coffee consumption and the risk of coronary heart disease,[1,4,5] such that the risk increases disproportionately to the increase in dose. Assuming caffeine contributes to the adverse health effects of coffee, a possible explanation for the nonlinear dose-response relationship is dose-dependent metabolism of caffeine.

The major route of caffeine metabolism is demethylation to dimethylxanthines and monomethylxanthines (Fig. 1).[8] Although animal studies suggest that the metabolism of caffeine may be dose-dependent,[9] dose-independent kinetics have been reported in several studies in human beings.[10,11] The possibility that the metabolism of caffeine in human beings might be dose-dependent is suggested by observations that the metabolism of theophylline (1,3,-dimethylxanthine and a minor metabolite of caffeine) can be saturable.[12,13] Because caffeine metabolism involves multiple demethylation steps, it is likely that competition for the same *N*-demethylation enzymes would occur, especially as metabolites accumulate with chronic dosing.

From the Division of Clinical Pharmacology and Experimental Therapeutics, Department of Medicine, University of California–San Francisco.

Supported by U.S. Public Health Service grant DA01696 from the National Institute on Drug Abuse and carried out in part in the General Clinical Research Center at San Francisco General Hospital Medical Center with support of the Division of Research Resources, National Institutes of Health (RR0-00083). Dr. Charles P. Denaro is a Merck International Fellow.

Received for publication March 1, 1990; accepted June 6, 1990.

Reprint requests: Neal L. Benowitz, MD, Division of Clinical Pharmacology and Experimental Therapeutics, San Francisco General Hospital Medical Center, Building 30, 5th Floor, 1001 Potrero Ave., San Francisco, CA 94110.

13/1/23004

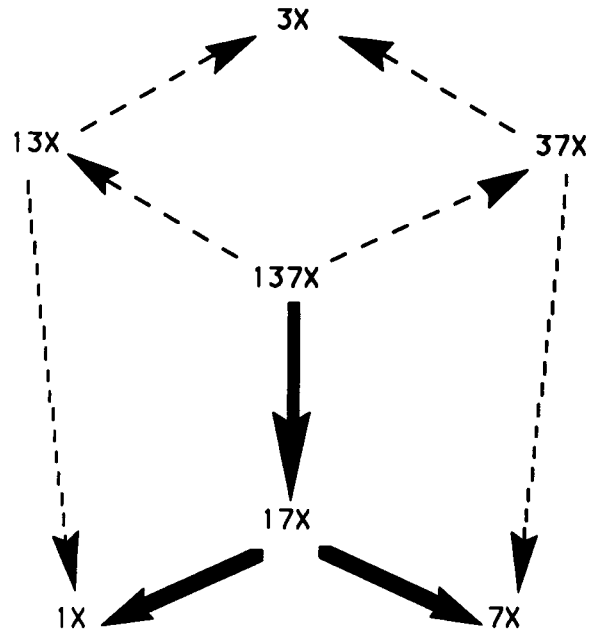

Fig. 1. *N*-Demethylation pathways of caffeine metabolism. Hydroxylation and acetylation pathways are not shown. Abbreviations are defined in the *legend* of Table I.

Our study was designed to investigate the hypothesis that the metabolism of caffeine is dose-dependent during repeated dosing when steady-state concentrations of caffeine and its metabolites have been achieved.

METHODS

Subjects

Nine healthy nonsmokers who were habitual coffee drinkers (4 or more cups per day) were admitted to the General Clinical Research Center at San Francisco General Hospital Medical Center for 16 days. There were seven men and two women, 19 to 55 years of age (mean age, 37 years). The body weights ranged from 55 to 99 kg (mean weight, 77 kg). On the basis of history, physical examination, electrocardiogram, and screening blood chemistry, subjects were healthy. Women had negative pregnancy tests before entry into the study. Subjects reported taking no medications (including oral contraceptive pills) and were instructed to abstain from methylxanthine consumption (coffee, tea, caffeinated soft drinks, and chocolate) for 1 week before admission. Written consent was obtained from each subject. The study was approved by the University of California, San Francisco, Committee on Human Research.

Experimental protocol

The study was conducted in three treatment blocks, each lasting 5 days. The sequence of treatment blocks was ordered using 3 × 3 Latin squares. Each patient participated in all three blocks. The blocks consisted of placebo, low-, or high-dose caffeine given in decaffeinated coffee to subjects every 2 hours, from 9 AM to 7 PM every day (total 6 cups per day; daily caffeine dose 0, 4.2, or 12 mg/kg/day). The coffee was prepared by adding anhydrous caffeine or nothing to decaffeinated instant coffee (Taster's Choice; 1 mg caffeine per serving) mixed with a constant volume of water. The diet was a standard hospital diet, with all methylxanthine-containing beverages and foods, as well as alcohol, prohibited.

At 2 PM on the third day of each block (when levels of caffeine and metabolites were expected to have reached steady-state), subjects received 25 mg of stable isotope-labeled caffeine ([2-^{13}C, 1,3,-$^{15}N_2$] caffeine) by intravenous injection. Blood was drawn before dosing and at 5, 15, 30, and 45 minutes and 1, 2, 3, 5, 8, 12, 24, and 36 hours after the dose. Two patients did not receive isotope-labeled caffeine because of unavailability of the labeled caffeine at the time of their admission. On the fifth day of each block, starting at 8 AM, blood samples were drawn for measurement of caffeine and its dimethylxanthine metabolites. Sampling times were 0, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 16, 20, and 24 hours. Urine was collected for this 24-hour time period for measurement of caffeine and caffeine metabolites.

Chemicals

All analytic standards were obtained from the Sigma Chemical Co. (St. Louis, Mo.), except those listed below. 1,7 Dimethyluric acid (17U) was obtained from Adams Chemicals (Round Lake, Ill.). Stable isotope-labeled (2-^{13}C, 1,3-$^{15}N^2$) caffeine was obtained from Cambridge Isotope Laboratories (Woburn, Mass.). Purification of this compound was accomplished by sublimation under reduced pressure. Sterile solution for injection was prepared by the Pharmaceutical Technology Laboratory at University of California, San Francisco. Caffeine-d_9, used as an internal standard for the labeled caffeine GCMS assay, was synthesized by methylation of xanthine with trideutero-methyl-iodide. AAMU (5-acetylamino-6-amino-3-methyluracil) was synthesized by the procedure of Khmelevskii et al.[14] modified by using 1 methyluric acid instead of uric acid. 1,8 Dimethylxanthine (18X) was obtained as a by-product in the synthesis of AAMU and proved to be a

Table I. Limits of quantification of plasma caffeine and dimethylxanthines and of urinary caffeine and metabolites based on three sets of quality control ($n = 6$)

	Quality control 1			*Quality control 2*			*Quality control 3*		
Compound	*Spiked conc*	*Measured conc**	*Coefficient of variation %*	*Spiked conc*	*Measured conc*	*Coefficient of variation %*	*Spiked conc*	*Measured conc*	*Coefficient of variation %*
Plasma									
Label-137X	0.010	0.008	15.0	0.020	0.017	7.0	0.10	0.10	3.6
137X	0.100	0.106	8.9	0.250	0.262	6.5	0.50	0.58	4.0
17X	0.100	0.101	5.3	0.250	0.235	2.3	0.50	0.47	0.9
37X	0.050	0.047	3.2	0.100	0.095	5.5	0.25	0.24	3.0
13X	0.050	0.049	11.2	0.100	0.100	2.8	0.25	0.24	4.3
Urine									
17X†	1.50	1.84	20.0	4.50	5.07	11.2	15.00	13.10	3.6
37X†	0.50	0.89	7.9	1.50	1.53	10.1	5.00	5.42	8.2
13X†	1.50	1.91	9.7	5.00	4.92	4.8	–	–	–
17U	1.50	1.57	13.7	4.50	4.67	5.2	15.00	15.60	3.2
13U	0.50	0.46	17.5	1.50	1.37	4.7	5.00	4.78	4.0
1X	2.50	2.65	11.2	7.50	7.19	5.1	75.00	72.80	2.4
3X	0.50	0.55	10.9	1.50	1.46	5.5	5.00	4.72	2.2
7X	4.50	3.24	15.2	15.00	13.70	10.3	–	–	–
1U†	7.50	8.86	3.0	75.00	75.60	2.8	–	–	–
3U	0.50	0.55	2.1	1.50	1.55	2.2	5.00	4.79	1.0
7U†	0.50	1.38	5.7	1.50	1.51	8.0	5.00	4.67	9.2
137U	1.50	1.80	14.5	5.00	1.80	6.1	–	–	–
AAMU	10.00	9.99	6.2	100.00	101.40	8.4	–	–	–

Where accuracy or variation was less than optimal with the lowest controls, values from these compounds were usually reported when they were greater than the second quality control. However, some of the data (22% or less) of these compounds fell between the two lowest controls. These values were only used when they were much greater (50% to 100%) than the lowest control.

The compounds: 137X, 1,3,7-trimethylxanthine (caffeine); Label-137X, 2-^{13}C, 1,3$^{15}N_2$ 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; AAMU, 5-acetylamino-6-amino-3-methyluracil.

*Concentrations are measured in mg/L, $n = 6$.

†Traces of peaks at the same retention times as these compounds in blank urine.

useful internal standard for the urinary caffeine metabolite HPLC assay.

Analytic methods

Isotope-labeled caffeine was measured by combined gas chromatography-mass spectroscopy (GC-MS) using caffeine-d_9 as the internal standard. Details of assay variability are provided in Table I. Plasma caffeine and dimethylxanthines were measured by HPLC using a modification of the method of Tang-Liu et al.[12] The limits of quantification, their associated coefficient of variation (CV), and abbreviations used for caffeine metabolites are listed in Table I. Traces of peaks at the same retention times as 137X, 13X, and 37X were evident in plasma from human subjects who had abstained from dietary methylxanthines for 1 week, so standard curves were prepared in a 5% aqueous solution of bovine albumin (which resulted in identical slopes as the human plasma).

Urinary caffeine was measured by GC using a 25 m 5% phenylmethyl-silicone capillary column and nitrogen-phosphorous detector. Coefficient of variation was 4.7%. Methylxanthines and methyluric acids in urine were measured by a modification of the method of Tang-Liu et al.[15] The most important modifications were the use of 18X as the internal standard instead of β-hydroxy ethyl theophylline (the latter interfered with 137U) and monitoring the methylxanthines at 270 nm (spectra for maximum absorption) and methyluric acids at 290 nm (maximum spectra) to aid in distinguishing metabolites and enhancing sensitivity. The ability to use computerized reintegration of peaks was also essential at low concentrations. The AFMU (5-acetylamino-6-formylamino-3-methyluracil) in each sample was converted to AAMU by adjusting the urine to pH 10 for 10 minutes.[16] AAMU was separated on a BioGel TSK-20 gel chromatography column (BIO-RAD, Richmond, Calif.) according to the method of Tang et al.[16] Table I lists the CV for the lower limit of quantification of each metabolite.

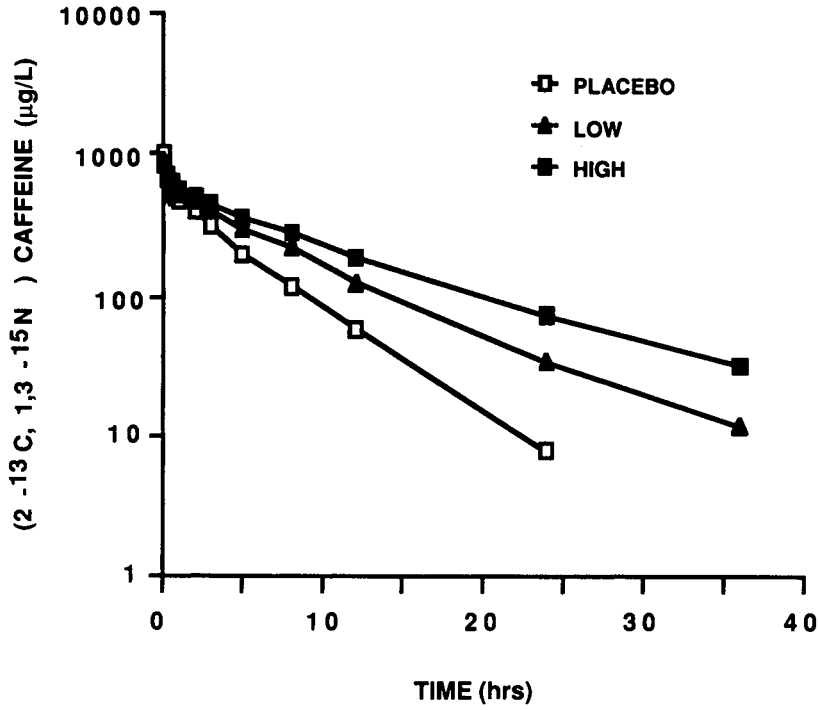

Fig. 2. Mean plasma concentrations of (2-^{13}C, 1,3-$^{15}N_2$) caffeine, 25 mg, given intravenously to subjects during differing caffeine consumption conditions: placebo, low caffeine dose (4.2 mg/kg/day), and high caffeine dose (12 mg/kg/day).

Table II. Pharmacokinetics of stable isotope caffeine during different caffeine-dosing conditions

	V_{ss} (L/kg)	CL (L/hr/kg)	Oral CL (L/hr/kg)	k_{el} (hr^{-1})	Half-life (hr)
Placebo†	0.57 ± 0.11	0.118 ± 0.049	–	0.19 ± 0.06	4.0 ± 1.4
Low	0.55 ± 0.08	0.069 ± 0.018†	0.068 ± 0.026	0.12 ± 0.03*	6.1 ± 1.6†
High	0.58 ± 0.10	0.054 ± 0.019†	0.057 ± 0.024	0.09 ± 0.03†	8.7 ± 2.3†‡

Data are mean values ± SD.
V_{ss}, Volume of distribution at steady state; CL, clearance; Oral CL, CL calculated from oral caffeine dose and concentrations; k_{el}, elimination rate constant.
*Placebo = decaffeinated coffee; low = 4.2 mg/kg caffeine/day; high = 12 mg/kg caffeine day.
†Significantly different from placebo ($p < 0.05$).
‡Significantly different from low ($p < 0.05$).

Pharmacokinetic analysis

Plasma caffeine data obtained after intravenous injection of the isotope-labeled compound were fitted to a two-compartment model equation using extended least-squares regression (MKMODEL). Data from three of the subjects did not demonstrate two-compartment kinetics, so we report data from this modeling on only the elimination rate constant (K_{el}) and half-life. Area under the plasma concentration-time curve (AUC) was computed by the linear trapezoidal rule, with the area extrapolated to infinity by dividing the last labeled isotope caffeine concentration by K_{el}. Steady-state volume of distribution (V_{ss}) was calculated using area under the moment curve[17] and clearance (CL) was calculated as Dose/AUC. Formation clearance (CL_f) of the major metabolite paraxanthine (17X) was approximated by using Ae_m/AUC[18] where Ae_m represents the total amount of 17X and its metabolites in urine collected for 24 hours (assuming steady state) and AUC is the AUC of caffeine. This equation is valid only if there is minimal first-pass effect, which is the case for oral caffeine.[19] The formation clearance is an approximation because some of the metabolites of 17X measured in the urine come from other pathways of caffeine metabolism.[8] However, because the fraction of caffeine metabolized to metabolites other than parax-

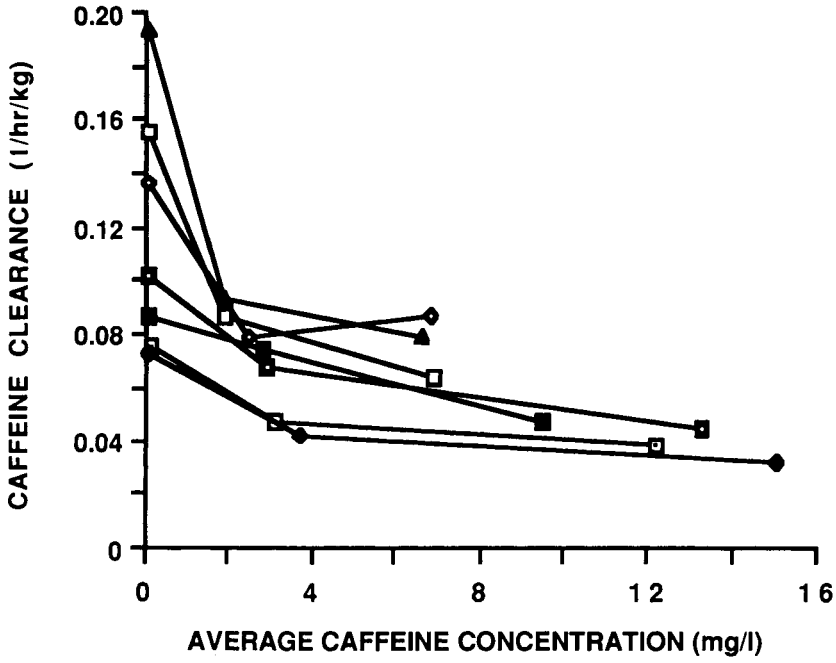

Fig. 3. The plasma clearance of labeled caffeine for each individual during each caffeine consumption condition plotted against the average caffeine concentrations over 24 hours for that condition.

anthine is so small,[20] the estimate of formation clearance of paraxanthine should be a good one. Metabolite clearance (CL_m) of 17X was calculated as $CL_m = CL_f * AUC/AUC_m$, in which AUC_m is the plasma AUC of 17X.[18]

Statistical analysis

Most of the data were analyzed using repeated measures analysis of variance (ANOVA). The Tukey post-test was used for multiple comparisons. Where only two conditions were compared, a two-tailed paired *t* test was used.

RESULTS

Fig. 2 shows mean log concentrations of labeled caffeine versus time after intravenous dosing during placebo, low-, or high-dose caffeine treatment blocks. (A plot of the median concentrations was similar.) This figure demonstrates a progressive reduction in the elimination rate and higher AUC of isotope-labeled caffeine as the dose of oral caffeine increased. Pharmacokinetic parameters are shown in Table II and Fig. 3. CL, k_{el}, and half-life were significantly different for low- and high-dose treatments when compared with placebo. The clearance of caffeine during the high-dose caffeine treatment block was 53% lower than during the placebo treatment block. Half-life was significantly different between low- and high-dose treatments. When CL of caffeine was calculated from the oral caffeine dose and its plasma concentrations, the results were almost identical to that calculated from the intravenous dose. As seen in Fig. 3, most of the decline in CL of caffeine with progressive doses occurs between placebo and the low-dose caffeine treatment.

The concentrations of caffeine and its dimethylxanthine metabolites throughout the day during oral dosing of caffeine are displayed in Fig. 4. With 6 cups of coffee given at regular intervals during the day, caffeine rises to a peak concentration at around 8 PM (1 hour after the last cup). During the day, there is less fluctuation in the concentrations of the dimethylxanthines than in the concentrations of caffeine. At a dose of 12 mg/kg/day of caffeine, 17X reaches a concentration of almost 6 mg/L. Table III tabulates the AUC of caffeine and dimethylxanthines for low- and high-dose treatments. Although the dose of caffeine was different by a factor of 2.9, when the low- and high-dose caffeine treatment blocks were compared, the AUC ratios for caffeine and its metabolites were all greater than 2.9. Both the CL_f and CL_m of 17X are significantly reduced during the high- compared with the low- caffeine dose treatment periods (Table IV). The percentage of the

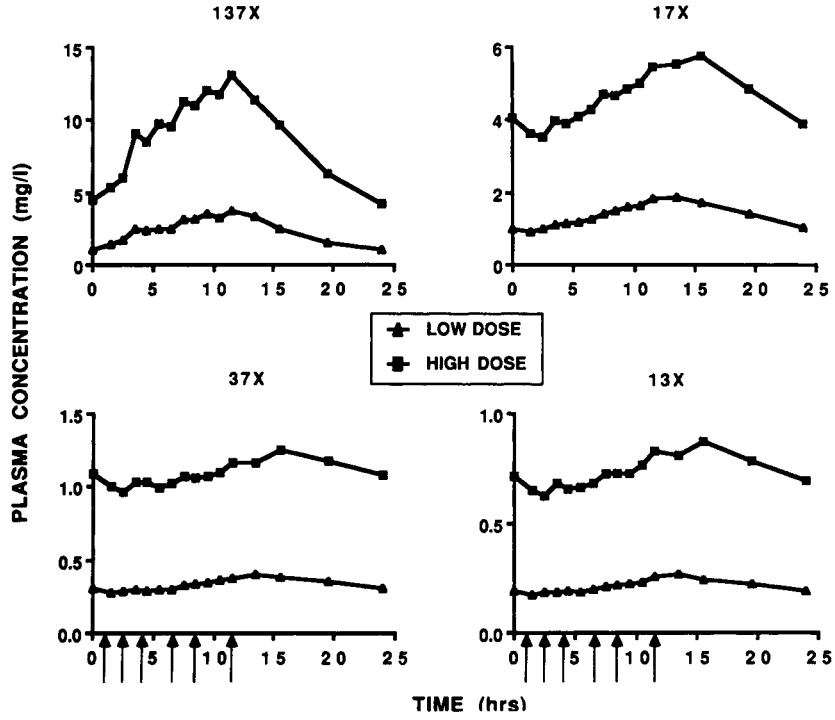

Fig. 4. Mean plasma concentrations of caffeine (137X), 1,7-dimethylxanthine, paraxanthine (17X), 3,7-dimethylxanthine, theobromine (37X), and 1,3-dimethylxanthine, theophylline (13X), measured over 24 hours during low and high caffeine consumption conditions.

Table III. AUC of plasma dimethylxanthines and caffeine during different caffeine-dosing conditions

	37X (mg · hr/L)	*13X (mg · hr/L)*	*17X (mg · hr/L)*	*137X (mg · hr/L)*
Low	8.1 ± 2.5	5.2 ± 1.6	33.6 ± 8.4	56.3 ± 21.0
High	26.7 ± 8.1	17.9 ± 6.7	112.6 ± 27.4	207.1 ± 78.9
AUC high / AUC low	3.3*	3.4*	3.4*	3.7*

Data are mean values ± SD.
See Table I for abbreviations.
*Significantly different from 2.9 ($p < 0.02$).

administered dose excreted as various metabolites during low- and high-dose treatment periods is shown in Table V. Total recovery fell from 57.9% to 45.5%; however, this did not quite reach statistical significance ($p = 0.08$). There were significant differences in the recovery of 1X, 1U, and 13U, consistent with inhibition of metabolism of dimethylxanthines to monomethylxanthines.

DISCUSSION

Our findings confirm the hypothesis that the metabolism of caffeine is dose-dependent under chronic dosing conditions. This dose-dependency was demonstrated even in the low-dose caffeine treatment condition (4.2 mg/kg/day), which is only moderately higher than the per capital daily caffeine consumption of 2.4 to 3.4 mg/kg.[21,22] Most of the decline in CL of infused labeled caffeine with progressively increasing doses of caffeine occurred between the placebo and low-dose conditions (see Fig. 3). Further investigation of dose-dependency at lower doses is warranted. A trend for a further fall (22%) in CL between the low- and high-caffeine dose was seen, but this did not reach statistical significance because of the relatively small numbers of subjects studied.

While dose-dependency of caffeine metabolism has

Table IV. Formation and metabolic clearance of paraxanthine during different caffeine-dosing conditions

	Low (L/hr/kg)	*High (L/hr/kg)*
CL_f	0.046 ± 0.033	0.027 ± 0.015*
CL_m	0.065 ± 0.037	0.041 ± 0.016*

Data are mean values ± SD.
CL_f, Formation clearance; CL_m, metabolite clearance.
*Significantly different from low ($p < 0.05$).

been demonstrated in the rat and rabbit,[9,23] previous studies of the clearance of caffeine in human beings were unsuccessful in confirming this.[10,11] Bonati et al.[10] studied single doses of caffeine up to 10 mg/kg. They studied only four subjects at five different doses, however, and only two of the subjects for the 10 mg/kg caffeine dose. A trend toward a decline in k_{el} and CL with increasing dose can be seen in the Bonati data. Newton et al.[11] studied six subjects given four different single doses. They also demonstrated a significant fall in k_{el} with increasing dose, though this seemed to be explained by a change in V_{area} rather than CL.

In contrast to single-dose studies, we examined the metabolism of caffeine while caffeine was dosed repeatedly, as it is consumed by most people. If dose-dependency results from end-product inhibition of demthylation, our experiment, conducted when caffeine and its dimethylxanthine metabolites were at steady-state conditions, would maximize the possiblity of competition for demethylation between caffeine and its metabolites. The use of stable isotope-labeled caffeine allowed us to characterize the disposition kinetics of caffeine in the face of ongoing consumption of caffeine. The clearance of caffeine can be measured as the ratio of daily dose/AUC caffeine taken orally at steady-state, but the method has the potential problems of missing rising caffeine concentrations after each cup of coffee (i.e., underestimating AUC) and the (unlikely) possibility that bioavailability might change with increasing doses or after chronic dosing. In fact, clearances calculated for low- and high-dose caffeine treatment periods using the oral caffeine data were nearly identical to the values obtained from intravenous doses of labeled caffeine.

In addition to the evidence of dose-related saturable metabolism of theophylline,[12,13] there is some evidence supporting dose-dependency of caffeine elimination in human beings. Using a breath test to measure *N*-demethylation of labeled caffeine, one group noted that the labeled CO_2 excretion rates plateaued as the dose of labeled caffeine increased from 3 to 5 mg/kg.[24] Tang-Liu et al.[25] showed that caffeine concentrations decayed in a nonlinear fashion, the shape of the concentration-time curve becoming convex descending at low concentrations, whereas the paraxanthine excretion rate plateaued at higher caffeine concentrations. Finally, in an overdose of an estimated 6 to 8 gm caffeine, the calculated elimination half-life was approximately 16 hours,[26] much longer than the half-life of caffeine in healthy subjects after lower doses of caffeine.

Table V. Urinary recovery of caffeine metabolites during different caffeine-dosing conditions*

Metabolite	*Low*	*High*
3U	†	0.04
3X	2.0	1.7
7U	1.7	1.2
7X	3.0	3.6
1U	14.8	10.2‡
1X	9.4	6.9‡
13U	1.5	1.0‡
13X	–	0.4
37X	1.2	1.3
17U	4.5	3.7
17X	4.7	3.6
137U	–	0.2
AAMU	14.0	10.3
137X	1.2	1.4
Total	57.9	45.5§

See Table I for abbreviations.
*Micromolar percentage of the dose given.
†Below lower limit of quantification.
‡Significantly different from low ($p < 0.05$).
§p Value = 0.08.

Because the major pathway of metabolism of caffeine, as well as its primary metabolites, is *N*-demethylation, it is likely that end-product inhibition of *N*-demethylation is the mechanism for dose dependency. Support for this idea comes from studies indicating that both theobromine and caffeine inhibit theophylline metabolism in rat liver slices[27]; that the coadministration of caffeine prolongs the elimination half-life of theophylline in human beings[28]; that the clearance of theobromine falls with exposure to other methylxanthines or with chronic dosing of theobromine[29]; that paraxanthine infusion reduces caffeine clearance in rabbits[23]; and that in human beings paraxanthine excretion rate plateaus as caffeine concentrations increase, as noted earlier.[25]

Our data support the idea of end-product inhibition. As caffeine clearance falls with increasing caffeine doses, the elimination clearance of paraxanthine falls

as well. The reduction in urinary excretion of the paraxanthine breakdown products, 1MX and 1MU, with the high-dose caffeine supports the idea that paraxanthine metabolism has been inhibited.

Although we measured most of the known metabolites of caffeine, urinary recovery of metabolites was incomplete and somewhat less than that reported in a few other studies.[8,10,25,30] While we did not measure some of the minor amino-methyluracil metabolites, these are thought to account for only 5% of the caffeine dose.[8] The differences between the urinary recovery findings of our study and other studies could be the result of methodologic problems in measuring urinary caffeine metabolites. We have been unable to find published data on sensitivity, specificity, and reproducibility of metabolite assays from other laboratories. In the absence of such data, the possbility that some metabolite concentations were overestimated by other investigators must be considered. In addition, urine recovery data from other studies were obtained after administration of single doses of caffeine. It is also possible that the pattern of metabolism of caffeine is different with chronic dosing, with generation of metabolites previously uncharacterized.

In conclusion, we have unequivocally demonstrated dose-dependency of caffeine metabolism in a chronic dosing situation. We present evidence that metabolism of paraxanthine, the major metabolite of caffeine, is saturable. While tolerance has been demonstrated for some of the pharmacologic actions of caffeine,[31] whether complete tolerance occurs when caffeine metabolism becomes nonlinear is unknown. We suggest that dose-dependency in the metabolism of caffeine and its metabolites, leading to disproportionate increases in blood levels with increasing daily doses of caffeine, may contribute to adverse effects of coffee that are seen at high levels of consumption.

We thank Clarissa Ramstead for supervising the inpatient admission for the subjects, Lisa Yu for assistance in laboratory analysis, and Gunnard Modin for guidance with the statistical analysis.

References

1. Williams PT, Wood PD, Vranizan KM, Albers JJ, Garay SC, Taylor CB. Coffee intake and elevated cholesterol and apolipoprotein B levels in men. JAMA 1985; 253:1407-11.
2. Davis BR, Curb JD, Borhani NO, Prineas RJ, Molteni A. Coffee consumption and serum cholsterol in the Hypertension Detection and Follow up Program. Am J Epidemiol 1988;128:124-36.
3. LaCroix AZ, Mead LA, Liang KY, Thomas CB, Pearson TA. Coffee consumption and the incidence of coronary heart disease. N Engl J Med 1986;315:977-82.
4. LeGrady D, Dyer AR, Shekelle RB, Stamler J, Liu K, Paul O, Lepper M, Shyrock AM. Coffee consumption and mortality in the Chicago Western Electric Company study. Am J Epidemiol 1987;126:803-12.
5. Rosenberg L, Werler MM, Kaufman DW, Shapiro S. Coffee drinking and myocardial infarction in young women: an update. Am J Epidemiol 1987;126:147-9.
6. Rosenberg L, Palmer JR, Kelly JP, Kaufman DW, Shapiro S. Coffee drinking and nonfatal myocardial infarction men under 55 years of age. Am J Epidemiol 1988;128:570-8.
7. Martin TR, Bracken MB. The association between low birth weight and caffeine consumption during pregnancy. Am J Epidemiol 1987;126:813-21.
8. Arnaud MJ, The pharmacology of caffeine. Prog Drug Res 1987;31:273-313.
9. Bonati M, Latini R, Tognoni G, Young JF, Garattini S. Interspecies comparison of in vivo caffeine pharmacokinetics in man, monkey, rabbit, rat and mouse. Metab Rev 1984;15:1355-83.
10. Bonati M, Latini R, Galletti F, Young JF, Tognoni G, Garatini S. Caffeine disposition after oral doses. CLIN PHARMACOL THER 1982;32:98-106.
11. Newton R, Broughton LJ, Lind MJ, Rogers HJ, Bradbrook ID. Plasma and salivary pharmacokinetics of caffeine in man. Eur J Clin Pharmacol 1982;21:45-52.
12. Tang-Liu DD, Williams RL, Riegelman S. Nonlinear theophylline elimination. CLIN PHARMACOL THER 1982; 31:358-69.
13. Efthimion H, Morgan DJ, Ioannides-Demos L, Raymond K, McLean AJ. Influence of chronic dosing on theophylline clearance. Br J Clin Pharmacol 1984;17:525-30.
14. Khmelevskii VI, Abramova EI, Variukhina LV. Preparation of theophylline and caffeine from uric acid. J Gen Chem USSR, Eng. Trans. 1958;28:2016-20.
15. Tang-Liu DD, Riegelman S. An automated HPLC assay for simultaneous quantitation of methylated xanthines and uric acids in urine. J Chromatogr Sci 1982;20: 155-9.
16. Tang BK, Zubovits T, Kalow W. Determination of acetylated caffeine metabolites by high-performance exclusion chromatography. J Chromatogr 1986;375:170-3.
17. Benet LZ, Galeazzi RL. Noncompartmental determination of the steady-state volume of distribution. J Pharm Sci 1979;68:1071-4.
18. Houston JB. Drug metabolite kinetics. Pharmacol Ther 1982;15:521-52.
19. Blanchard J, Sawers SJA. The absolute bioavailability of caffeine in man. Eur J Clin Pharmacol 1983;24:93-8.
20. Lelo A, Miners JO, Robson RA, Birkett DJ. Quantitative assessment of caffeine partial clearances in man. Br J Clin Pharmacol 1986;22:183-6.
21. Council on Scientific Affairs. Caffeine labeling. JAMA 1984;252:803-6.

22. Shirlow MJ. Patterns of caffeine consumption. Hum Nutr Appl Nutr 1983;37A:307-13.
23. Dorrbecker SH, Ferraina RA, Dorrbecker BR, Kramer PA. Caffeine and paraxanthine pharmacokinetics in the rabbit: Concentration and product inhibition effects. J Pharmacokin Biopharm 1987;15:117-32.
24. Kotake AN, Schoeller DA, Lambert GH, Baker AL, Schaffer DD, Josephs H. The caffeine CO_{l2} breath test: Dose response and route of N-demethylation in smokers and nonsmokers. Clin Pharmacol Ther 1982;32:261-9.
25. Tang-Liu DD, Williams RL, Riegelman S. Disposition of caffeine and its metabolites in man. J Pharmacol Exp Ther 1983;224:180-5.
26. Leson CL, McGuigan MA, Bryson SM. Caffeine overdose in an adolescent male. J Tox Clin Tox 1988;26:407-15.
27. Lohman SM, Meich RP. Theophylline metabolism by the rat microsomal system. J Pharmacol Exp Ther 1976;196:213-25.
28. Monks TJ, Caldwell J, Smith RL. Influence of methylxanthine-containing foods on theophylline metabolism and kinetics. CLIN PHARMACOL THER 1979;26:513-24.
29. Drouillard DD, Vesell ES, Dvorchik BH. Studies on theobromine disposition in normal subjects. CLIN PHARMACOL THER 1978;23:296-302.
30. Blanchard J, Sawers JA, Jonkman JHG, Tang-Liu DD. Comparison of the urinary metabolite profile of caffeine in young and elderly males. Br J Clin Pharmacol 1985;19:225-32.
31. Robertson D, Wade D, Workman R, Woosley RL, Oates JA. Tolerance to the humoral and hemodynamic effects of caffeine in man. J Clin Invest 1981;67:1111-7.