PHARMACOEPIDEMIOLOGY AND DRUG UTILIZATION

Racial and gender differences in *N*-acetyltransferase, xanthine oxidase, and CYP1A2* activities

Background: The urinary molar concentration ratios of several caffeine metabolites are indicators of specific drug metabolizing enzyme activities. The ratios of 5-acetyl-amino-6-formylamino-3-methyluracil (AFMU) to 1-methylxanthine (1X), AFMU to 1X plus 1-methyluric acid (1U), and AFMU to 1X + 1U + AFMU are indicative of *N*-acetyltransferase (NAT) activity; the ratios of 1U to 1X and 1U to 1U + 1X indicate xanthine oxidase activity; and the ratio of the sum of 7-demethylated metabolites (AFMU + 1X + 1U) to the precursor for all three compounds, paraxanthine (PX), is a putative indicator of CYP1A2 oxidative activity. Our objective was to discern whether there are race-, gender-, and age-related differences in these indexes of drug-metabolizing activity.

Methods: In 342 normal healthy unrelated subjects, metabolites were measured in urine collected after administration of low-dose caffeine.

Results: By two-way analysis of variance, NAT activity was higher in black subjects than in white subjects when assessed as AFMU/(1U + 1X) or as AFMU/(AFMU + 1U + 1X) ($p = 0.001$ and $p = 0.002$, respectively), but less so by use of AFMU/1X ($p = 0.08$). Xanthine oxidase activity, as assessed by 1U/1X or as 1U/(1U + 1X), was lower in black subjects than in white subjects ($p = 0.02$ and $p = 0.001$, respectively) and was lower in males than in females ($p = 0.001$ for both ratios). Females had higher AFMU/1X ratios ($p = 0.03$) because of higher xanthine oxidase activity. In a model in which AFMU/1X was the dependent variable and race, sex, age, and an index of xanthine oxidase (1U/1X) were independent variables, only race and 1U/1X were significant determinants of this NAT index ($p = 0.003$ and $p < 0.001$, respectively). The CYP1A2 ratio was lower in black subjects ($p = 0.036$) and in females ($p = 0.015$).

Conclusion: Racial and gender differences in xanthine oxidase activity render the AFMU/1X ratio less reliable as an assessment of NAT activity in a heterogeneous population compared with the AFMU/(1U + 1X) or AFMU/(AFMU + 1U + 1X) ratios. The observed racial and gender differences in NAT, xanthine oxidase, and CYP1A2 activities may have implications for racial and gender differences in drug effects and carcinogen biotransformation. (CLIN PHARMACOL THER 1992;52:643-58.)

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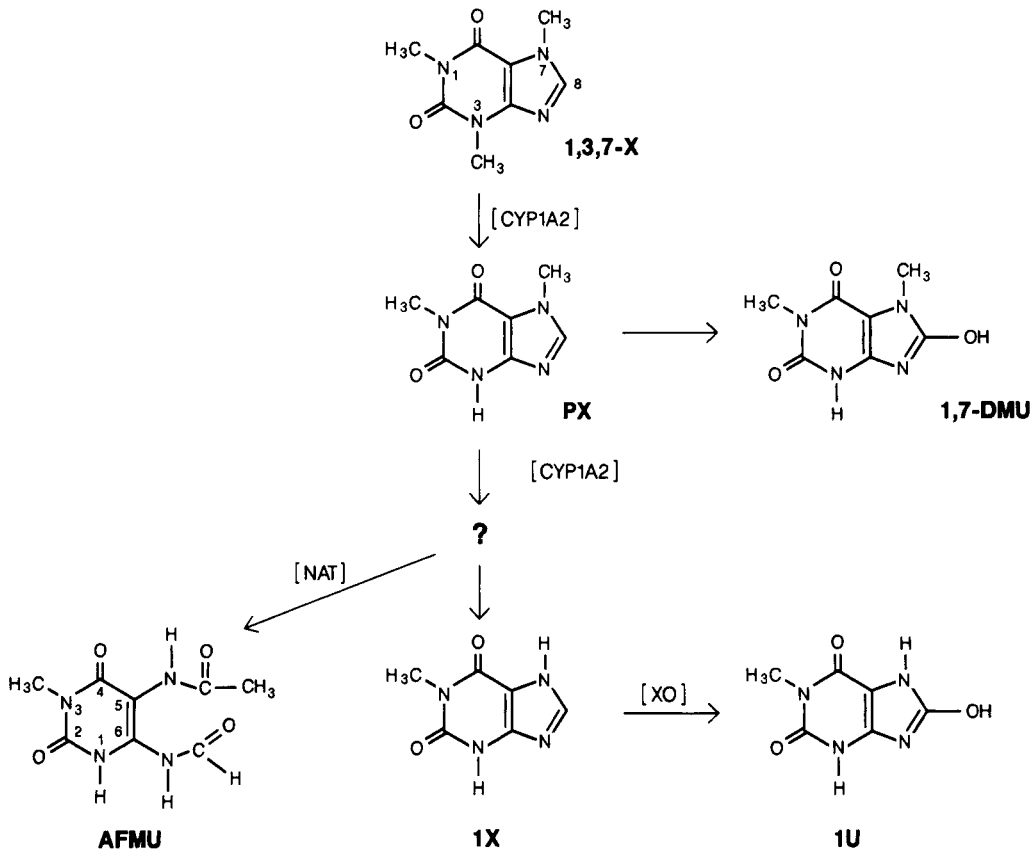

Fig. 1. Simplified metabolic scheme for caffeine (1,3,7-X). AFMU, 5-Acetyl-amino-6-formyl-amino-3-methyluracil; 1X, 1-methylxanthine; 1U, 1-methyluric acid; PX, paraxanthine; 1,7-DMU, 1,7-dimethyluric acid; NAT, *N*-acetyltransferase; XO, xanthine oxidase; CYP1A2, cytochrome P4501A2.

Interindividual variability in drug metabolism has been shown to translate into variability in drug efficacy and toxicity. The identification of factors that determine or correlate with drug-metabolizing enzyme activities may therefore assist in accounting for variation in drug response. Several important drug metabolizing enzymes are involved in the primary, secondary, and tertiary metabolism of caffeine (Fig. 1). *N*-Acetyltransferase (NAT) catalyzes the acetylation of an intermediate in the demethylation of paraxanthine to 5-acetyl-amino-6-formylamino-3-methyluracil (AFMU);[1] xanthine oxidase catalyzes the oxidation of 1-methylxanthine (1X) to 1-methyluric acid (1U);[2,3] and CYP1A2 catalyzes the 3-demethylation of caffeine[4] and is also thought to catalyze the 7-demethylation of paraxanthine (PX).[5-8] Therefore the ratios of urinary concentrations of metabolites after an oral dose of caffeine have been used as in vivo indicators of NAT, xanthine oxidase, and CYP1A2 activities in several studies. However, there have not been previous comparative studies of caffeine as an indicator of drug metabolizing enzymes in an American population of white subjects and black subjects.

The genetic polymorphism in NAT activity was described more than 30 years ago, and isoniazid was the first known substrate.[9-10] Since that time, more than 25 additional substrates for NAT have been identified,[11] and the biochemical and molecular bases of the polymorphism have been elucidated.[12-15] This polymorphism has implications not only for the adverse effects and efficacy profiles of affected drug substrates[16-18] but also for risk of cancer because the prevalences of several cancers have been associated with low NAT activity.[19-21] Urinary concentration ratios of metabolites after an oral dose of caffeine have been used as safe and useful indicators of NAT phenotype in various white and Oriental populations,[18,22-25] despite potential analytic pitfalls.[26] The specific ratios

that have been used include AFMU/1X,[8,22-23,25,27-29] AFMU/(AFMU + 1U + 1X),[16] and similar ratios in which AFMU has been converted to its degradation product, 5-acetyl-amino-6-amino-3-methyluracil (AAMU).[18,30,31] Two relatively large scale studies of NAT phenotype in American white subjects and black subjects have been reported,[9,32] and both relied on serum concentrations of isoniazid and its acetylated metabolite to indicate NAT phenotype. No difference in the prevalence of fast and slow acetylators in white subjects compared with black subjects was reported,[9,32] although there have been uncontrolled reports in African black subjects of higher,[33-35] lower,[36-38] and similar[39] prevalences of fast acetylator phenotype, compared with that reported for white populations.[11]

Xanthine oxidase is a cytosolic enzyme responsible for the oxidation of several endogenous compounds (e.g., catabolism of hypoxanthine to xanthine and then to uric acid), as well as several important drugs (e.g., mercaptopurine and azathioprine). The use of caffeine as an indicator of xanthine oxidase activity has been shown in work that demonstrated the effects of allopurinol on urinary 1U/1X ratios[2,3] and the demonstration that 1U/1X urinary ratios correlated with urinary concentration ratios of uric acid to xanthine and hypoxanthine.[2] Because xanthine oxidase is necessary to catalyze essential constitutive reactions, it is not surprising that its activity does not appear to be polymorphic.[3,6,40] However, others have reported an approximate 3.9-fold range in xanthine oxidase activity among individuals as assessed by in vitro liver cytosolic conversion of xanthine to uric acid[40] and a 2.2-fold variation in vivo as assessed by the 1U/(1U + 1X) ratio after caffeine administration.[6] Moreover, gender differences in xanthine oxidase activity have been previously reported.[40] Racial differences between black subjects and white subjects in xanthine oxidase activity have not been shown.

In humans, aryl hydrocarbon hydroxylase activities are mediated primarily by constitutive CYP1A2 and the inducible CYP1A1 cytochromes P450.[41] These enzymes are responsible for the metabolism of several drugs (e.g., phenacetin and theophylline) and a large number of polycyclic aromatic hydrocarbons, including potent carcinogenic substances.[41] The relationship between cancer risk and CYP1A1 and CYP1A2 activities is probably complex, because many carcinogenic hydrocarbons are inactivated by these P450s, but they also induce CYP1A1 and CYP1A2 expression, and some hydrocarbons are bioactivated to more reactive species in the process of biotransformation by the enzymes they induce.[42] The usefulness of caffeine as a tool to assess CYP1A2 activity is controversial, partly because of the lack of a "gold standard" for assessing CYP1A2 activity in vivo. Exhalation of 3-^{13}C-methyl-labeled caffeine as $^{13}CO_2$,[43-45] a urinary ratio of (AFMU + 1U + 1X) to 1,7-dimethyluric acid (1,7-DMU),[5-7] a urinary ratio of PX/caffeine,[28,46] and (AFMU + 1U + 1X)/PX[8,25] have all been proposed as indicators of CYP1A2 activity. These measures would presumably also reflect the degree of CYP1A1 activity. Several of these indicators have been shown to be increased in smokers compared with nonsmokers,[45,46] as well as increased in subjects exposed to aromatic hydrocarbon pesticides.[44] Racial differences in CYP1A2 indexes between Oriental and white subjects have been reported,[8,25] although differences between black subjects and white subjects have not.[43] The data in this article help to establish a baseline for the usual range of values, as well as race and gender influences, for (AFMU + 1U + 1X)/PX as an indicator of the CYP1A1 and CYP1A2 P450s.

The safety, availability, and ease of administration of caffeine have made it possible to perform large population studies in which drug-metabolizing enzyme activities can be assessed and compared between different subgroups of subjects. Our purpose was to determine whether and the extent to which these drug-metabolizing enzyme activities differ by race, gender, and age.

METHODS

Subjects were unrelated normal healthy volunteers. Children were enrolled from local private elementary and secondary schools; adults were enrolled from the staff of St. Jude Children's Research Hospital (Memphis, Tenn.). The protocol was approved by the hospital's institutional review board for clinical trials. Informed parental consent was obtained for all subjects younger than 18 years of age; the consent of the subject was also obtained for those older than 12 years of age. A complete medication history for the previous 2 weeks and basic demographic and family pedigree data were collected. Smoking, passive smoking, dietary, and occupational histories were not recorded. Excluded from this analysis were those with any chronic disease, children younger than 2 years of age, related individuals, and those of ethnic origin other than white or black. Subsets of subjects were generated for each of the six metabolic ratios by the following procedure. First, ratios were excluded from analysis if the subject had taken a medication known or suspected to induce, inhibit, or be substrates for

Table I. Race and gender of subjects randomly chosen for inclusion in each set of metabolic ratios

	Black males	*Black females*	*White males*	*White females*
309 Subjects included in all 6 ratios	31	32	117	129
AFMU/1X	35	41	121	145
AFMU/(1U + 1X)	37	39	121	144
AFMU/(AFMU + 1U + 1X)	37	39	121	144
1U/1X	37	38	121	144
1U/(1U + 1X)	37	38	121	144
(AFMU + 1U + 1X)/PX	35	36	122	138

AFMU, 5-Acetyl-amino-6-formylamino-3-methyluracil; 1X, 1-methylxanthine; 1U, 1-methyluric acid; PX, paraxanthine.

CYP1A2, xanthine oxidase, or NAT within 2 weeks of the study. Second, if a subject was studied on more than one occasion, the result used in the current analysis was chosen randomly by use of a subject-specific random number generator. Third, if two or more subjects were found to be related to each other, one subject from the family was chosen randomly by use of a family-specific random number generator.

After collection of a baseline urine sample, caffeine (>23 mg) was administered orally as at least one-half can of caffeine-containing carbonated beverage as described previously.[47] Urine was collected over the next 4 hours, and a 30 ml aliquot was immediately poured into a urine cup that contained 700 mg ascorbic acid. Aliquots were frozen and later thawed and extracted and assayed in duplicate by HPLC with ultraviolet detection for the caffeine metabolites AFMU, 1X, 1U, and PX as described previously.[47] Sources of metabolite standards were as follows: AFMU, provided by Dr. B. K. Tang, Toronto, Ontario, Canada; 1U, Fluka, Ronkonkoma, N.Y.; 1X, Pfaltz & Bauer, Waterbury, Conn.; and PX, Aldrich, Milwaukee, Wis. Interday and intraday coefficients of variation (CV) for all four metabolites were <10%; limits of sensitivity were approximately 0.4 nmol on the column for all metabolites.

Enzyme activities were assessed with urinary metabolic ratios. The ratios were calculated by taking the ratio of the averaged duplicate measurements of molar concentrations of AFMU, 1X, 1U, and PX. Because the distributions of all of the ratios were highly skewed, natural logarithms (log) of ratios were used for most of the statistical analyses. Coefficients of variation were estimated as the ratio of the sample standard deviation to the sample mean.

To assess whether enzyme activities were affected by age, plots of metabolic ratios versus age, and linear regression of natural logs of ratios regressed on age were performed. χ^2 Tests were used for comparisons of proportions of subjects between racial and gender groups. Linear association between logs of ratios were assessed by use of Pearson correlation coefficients. Two-way analysis of variance (ANOVA) was performed to examine the effect of race and gender on the natural logs of the ratios and on metabolite concentrations. To determine whether frequency distributions of natural logs of NAT ratios were fitted better by a mixture of two normal distributions or by a single normal distribution, likelihood ratio tests were used. Maximum likelihood estimators for the means and standard deviations and the proportion of the mixture were solved iteratively.[48]

RESULTS

Demographic characteristics for the subjects randomly selected for each ratio are summarized in Table I. There were no significant differences in the mean ages (by a two-sample t test) or in the proportion of female subjects (by a χ^2 test) between the two racial groups for any of the six ratios analyzed.

The mean values of each metabolite concentration and the sums of various metabolite concentrations, within each race and gender, are summarized in Table II. Males had significantly more concentrated urine than females, with every metabolite being present in greater concentrations. There were no racial differences in the concentration of any metabolite.

Correlations among natural logs of metabolite concentrations are indicated in Table III. Concentrations of all individual metabolites were highly correlated with each other, particularly 1U with 1X concentrations ($r = 0.90$). Correlations between ratios that assess the same enzyme activity [e.g., log 1U/1X and log 1U/(1U + 1X)] were also highly correlated with each other (i.e., $r > 0.95$). However, with the exception of the two xanthine oxidase ratios and log AFMU/1X, in which correlations were 0.35 and 0.31, the correlations between ratios that assessed different enzymes were all not significantly different from zero (all with p value > 0.14).

Table II. Mean values of metabolite concentrations by race and gender

Metabolite	*Black subjects (n = 63)*	*White subjects (n = 246)*	*p Value*	*Females (n = 161)*	*Males (n = 148)*	*p Value*
Log AFMU (μmol/L)	2.35	2.10	0.153	2.01	2.29	0.049
Log 1U (μmol/L)	3.36	3.54	0.185	3.34	3.68	0.002
Log 1X (μmol/L)	3.13	3.21	0.549	2.95	3.46	<0.001
Log PX (μmol/L)	2.32	2.31	0.897	2.19	2.44	0.006
Log (1U + 1X) (μmol/L)	3.99	4.10	0.405	3.88	4.29	<0.001
Log (AFMU + 1X + 1U) (μmol/L)	4.21	4.28	0.574	4.07	4.47	<0.001
Log (AFMU + 1X + 1U + PX) (μmol/L)	4.36	4.42	0.634	4.23	4.61	<0.001

Table III. Correlations among metabolite concentrations and ratios in 309 subjects

	Log AFMU	*Log 1U*	*Log 1X*	*Log PX*	*NAT^1*	*NAT^2*	*NAT^3*	*XO^1*	*XO^2*
Log 1U	0.692								
Log 1X	0.662	0.902							
Log PX	0.693	0.788	0.807						
NAT^1	0.443	−0.086	−0.173	−0.004					
NAT^2	0.468	−0.097	0.128	0.032	0.961				
NAT^3	0.450	−0.098	−0.126	0.032	0.956	0.997			
XO^1	0.025	0.035	−0.183	−0.097	0.349	0.084	0.079		
XO^2	0.033	0.055	−0.157	−0.066	0.311	0.066	0.063	0.971	
CYP1A2	0.268	0.368	0.346	0.030	−0.050	−0.070	−0.080	0.045	0.036

NAT, *N*-Acetyltransferase; XO, xanthine oxidase.
NAT^1 = log AFMU/1X; NAT^2 = log AFMU/(1U + 1X); NAT^3 = log AFMU/(AFMU + 1U + 1X); XO^1 = log 1U/1X; XO^2 = log 1U/(1U + 1X); CYP1A2 = log (AFMU + 1U + 1X)/PX.

The CVs of the natural logs of each ratio (and of the untransformed ratios) in the 309 subjects who had all six ratios evaluable were as follows: AFMU/1X, −90% (109%); AFMU/(1U + 1X), −46% (97%); AFMU/(AFMU + 1U + 1X), −35% (74%); 1U/1X, 148% (58%); 1U/(1U + 1X), −36% (18%); and (AFMU + 1U + 1X)/PX, 28% (85%).

NAT activity indexes

AFMU/1X. AFMU/1X data were available from a total of 266 white subjects and 76 black subjects. The median age was 11.8 years for the black males (age range, 3.8 to 46.0 years), 11.2 years for black females (age range, 5.2 to 41.0 years), 11.2 years for white males (age range, 2.9 to 42.4 years), and 12.3 years for white females (age range, 3.6 to 45.5 years).

The log of the AFMU/1X ratio was examined as both a continuous variable and an indicator with which to divide subjects into two discrete phenotypes. This ratio was the most variable of the NAT ratios in the population, with an overall CV of −90% in the entire population. In a linear regression model with race, sex, and age as regressors, age had no influence on log AFMU/1X ($p = 0.91$). Two-way ANOVA indicated no evidence of an interaction between race and sex ($p = 0.24$). It also indicated that only female sex ($p = 0.03$) and not black race ($p = 0.08$) was significantly associated with higher log AFMU/1X ratios at an α level of 5% (Table IV). The mean of log AFMU/1X ratios was −0.87 in black males, −0.87 in black females, −1.24 in white males, and −0.95 in white females. Therefore most of the effect of gender on the AFMU/1X ratio comes from white subjects, and the racial difference in female subjects ($p = 0.62$) is smaller than that in male subjects ($p = 0.04$).

As an indicator of phenotype, the estimated antimode for the log AFMU/1X ratio in all 342 subjects was −0.839, that is, log(0.432), assuming the frequency distribution is a mixture of two normal distributions, which is in close agreement with that described in white populations by other investigators.[8] With this cutpoint, 97 of 266 (37%) white subjects and 37 of 76 (49%) black subjects were fast acetylators ($p = 0.054$ by a χ^2 test). However, an examination of the frequency distribution of this ratio (Fig. 2) indicates a different distribution of ratios in white subjects than in black subjects. Therefore, separate antimodes were estimated in white subjects and black sub-

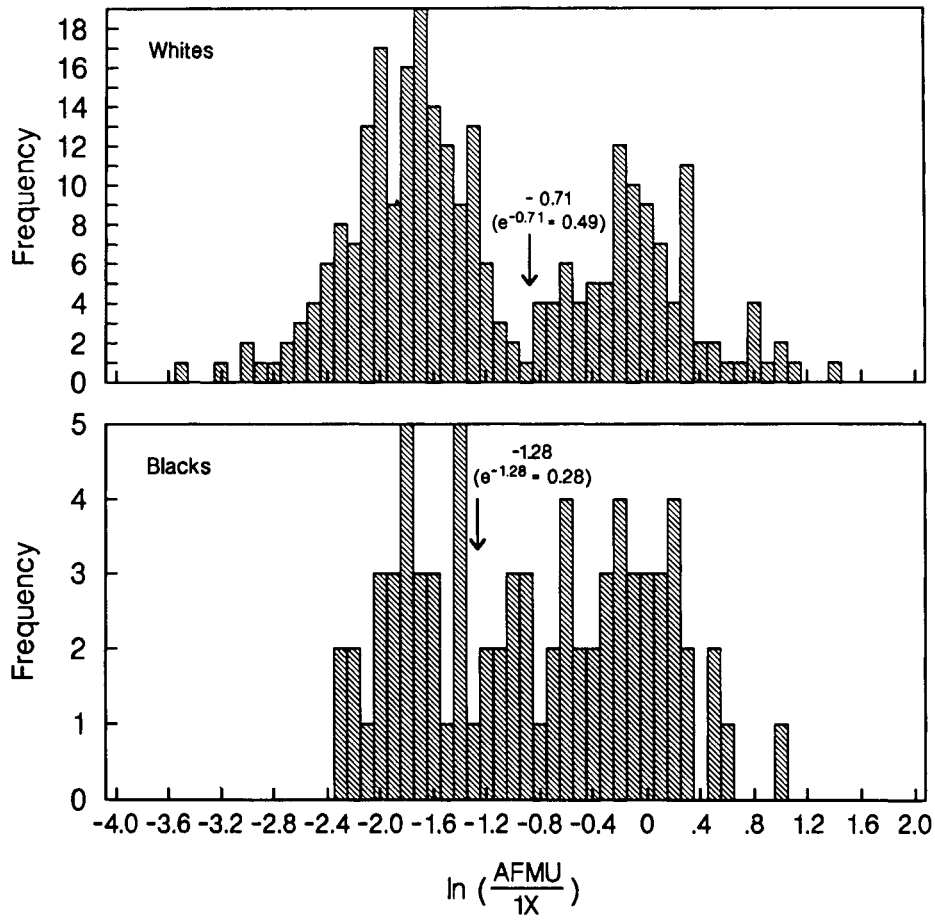

Fig. 2. Frequency distribution of log AFMU/1X in black subjects (n = 76) versus white subjects (n = 266), with maximum likelihood estimates of antimodes indicated.

Table IV. ANOVA for race and gender effects on caffeine metabolic ratios

Ratio	*n*	*p Value for race (white versus black)*	*p Value for gender (male versus female)*	*Overall p value for ANOVA*
Log AFMU/1X	342	0.08 (W < B)	0.03 (M < F)	0.03
Log AFMU/(1U + 1X)	341	0.001 (W < B)	0.16	0.005
Log AFMU/(AFMU + 1U + 1X)	341	0.002 (W < B)	0.15	0.003
Log 1U/1X	340	0.02 (B < W)	0.001 (M < F)	<0.001
Log 1U/(1U + 1X)	340	0.001 (B < W)	0.001 (M < F)	<0.001
Log (AFMU + 1U + 1X)/PX	331	0.06 (B < W)	0.03 (F < M)	0.02
Log[log (AFMU + 1U + 1X)/PX]	331	0.036 (B < W)	0.015 (F < M)	0.007

W, White; B, black; M, male; F, female.

jects, assuming that the frequency distributions for each group were mixtures of two normal distributions. Likelihood ratio tests indicated that a bimodal distribution better described the data than a unimodal distribution in both black subjects (p = 0.0016) and white subjects (p < 0.0001). With use of maximum likelihood estimates to determine the antimodes in white subjects (log 0.49 = −0.71) and in black subjects (log 0.28 = −1.28), we observed 36% fast acetylators in white subjects and 61% fast acetylators in

black subjects. This was a significant difference in the acetylator phenotype prevalence between races by the χ^2 test ($p < 0.0001$). The difference between the prevalences of the two acetylator phenotypes was greater than the corresponding difference when the same antimode had been used for both groups. There tended to be more female fast acetylators in white subjects ($p = 0.06$), whereas there were no gender differences in phenotype prevalences in black subjects ($p = 0.87$).

Because the frequency distribution of log AFMU/1X in black subjects was different than in white subjects, we hypothesized that racial differences in xanthine oxidase activity were affecting the distribution of the log AFMU/1X ratio in black subjects. To assess the effect of xanthine oxidase activity on the log AFMU/1X ratio, log 1U/1X ratio (as the xanthine oxidase index), race, and sex were examined as independent variables in a model with log AFMU/1X as the dependent variable. In this model, only race and log 1U/1X ($p = 0.003$ and $p < 0.001$, respectively), and not gender ($p = 0.31$), were significant predictors of log AFMU/1X.

AFMU/(1U + 1X). This ratio was a less variable index of NAT activity than AFMU/1X, with a CV in the population of −46%. Again, age had no influence on log AFMU/(1U + 1X). In two-way ANOVA, only black race was significantly positively associated ($p = 0.003$) with the log AFMU/(1U + 1X) ratio; female sex was no longer significant ($p = 0.16$; Table IV). To assess the effect of xanthine oxidase activity on the AFMU/(1U + 1X) ratio, log 1U/1X ratio (as a xanthine oxidase index), race, and sex were examined as independent variables in a model with log AFMU/(1U + 1X) as the dependent variable. In this model, race was a significant predictor ($p = 0.002$), and log 1U/1X was less so ($p = 0.059$); gender was not a significant predictor ($p = 0.33$).

The frequency distribution of log AFMU/(1U + 1X) as an NAT index was again different in white subjects than in black subjects (Fig. 3). However, as opposed to log AFMU/1X, essentially the same antimode was estimated in white subjects (−1.44) as in black subjects (−1.43). However, a likelihood ratio test showed that a bimodal distribution did not fit the data significantly better than a unimodal in black subjects ($p = 0.255$), whereas a bimodal fit was better in white subjects ($p < 0.0001$). These antimodes result in a 30% and 46% prevalence of fast acetylators ($p = 0.01$) in white subjects and black subjects, respectively.

AFMU/(AFMU + 1U + 1X). This NAT index, as expected, was the least variable of the NAT indexes, with a CV of −35%. Again, age had no significant effect on log AFMU/(AFMU + 1U + 1X). In two-way ANOVA, only black race was significantly positively associated ($p = 0.002$) with this NAT index; female sex was not significant ($p = 0.15$; Table IV). To assess the effect of xanthine oxidase activity on the log AFMU/(AFMU + 1U + 1X) ratio, log 1U/1X ratio (as the xanthine oxidase index), race, and sex were examined as independent variables in a model with log AFMU/(AFMU + 1U + 1X) as the dependent variable. In this model, race was a significant predictor ($p = 0.001$), and log 1U/1X was less so ($p = 0.07$); gender was not significant ($p = 0.30$).

Fig. 4 indicates that the antimodes in white subjects and black subjects are similar (−1.59 and −1.63, respectively); the assumption of a bimodal distribution yielded a significantly better fit than a unimodal fit for both black subjects ($p = 0.039$) and white subjects ($p < 0.0001$). Again, there were more black subjects with fast acetylation activity (42%) than white subjects (28%); ($p = 0.015$).

Xanthine oxidase indexes

1U/1X. Age was not correlated with log 1U/1X ($p = 0.66$). This index of xanthine oxidase activity was highly variable, with a CV of 148%, although it was primarily a small number of outliers that accounted for this large CV. Both white race ($p = 0.02$) and female sex ($p = 0.001$) were positively associated with xanthine oxidase activity (Table IV). As can be seen in Figs. 5 and 6, the lowest xanthine oxidase activities were observed in black males, and the highest was observed in females of both races. These distributions did not appear to be bimodal.

1U/(1U + 1X). By use of the 1U/(1U + 1X) ratio, the population interindividual variation in xanthine oxidase activity was reduced, so that the CV in the population was only −36%. Both white race ($p = 0.001$) and female sex ($p = 0.001$) were positively associated with log 1U/(1U + 1X) (Table IV). Age was not significantly correlated ($p = 0.37$). The frequency distributions of this index (Fig. 7) indicate more clearly than the log 1U/1X ratio that it is primarily a few males with very low xanthine oxidase activities that account for the deviation from a log normal distribution.

CYP1A2 index

(AFMU + 1U + 1X)/PX. The frequency distribution for this index of CYP1A2 activity was highly

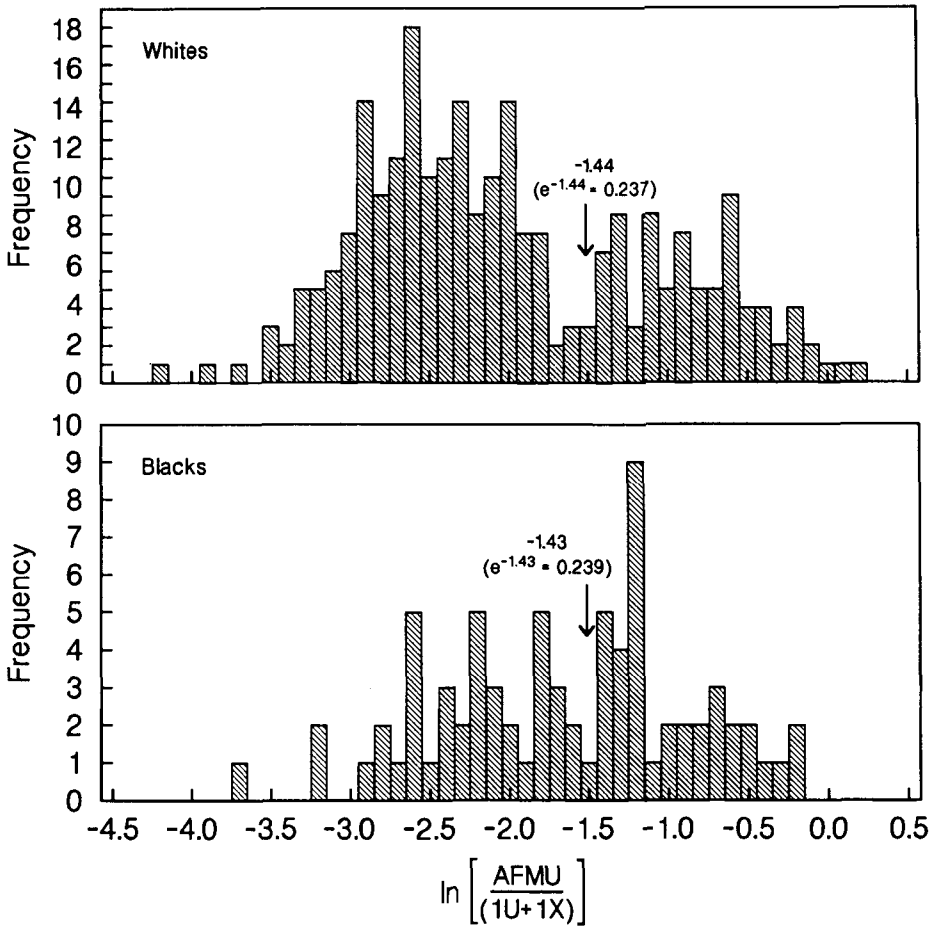

Fig. 3. Frequency distribution of log AFMU/(1U + 1X) in black subjects (n = 76) versus white subjects (n = 265), with maximum likelihood estimates of antimodes indicated.

skewed (Figs. 8 and 9). The natural log of the (AFMU + 1U + 1X)/PX ratio had a CV of 28% in this population. Because the frequency distribution of the log (AFMU + 1U + 1X)/PX still results in a skewed distribution, double logs were taken of the untransformed ratios to have a more symmetric distribution. Although the p value for the regression coefficient of age was 0.007, age is probably not a clinically significant factor because it accounts for such a low amount of the variability in the ratio, with an R^2 = 2.2%. By two-way ANOVA, white race (p = 0.036) and male sex (p = 0.015) were both significantly associated with higher CYP1A2 indexes (Table IV).

DISCUSSION

This represents the first comparative evaluation in American black subjects and white subjects of drug-metabolizing enzyme activities assessed by caffeine metabolic ratios. It is necessary to understand the effects of various demographic variables on caffeine metabolic ratios to compare enzyme indexes between patient subsets composed of individuals with diverse gender and racial backgrounds. We have found differences between black subjects and white subjects in all three enzyme activities assessed by caffeine: *N*-acetyltranferase, xanthine oxidase, and CYP1A2. Moreover, we have found gender differences in xanthine oxidase and CYP1A2 activities. However, in the process of defining these demographic differences some important limitations in one of the most widely used NAT indexes, the AFMU/1X ratio, have been illuminated.

Extensive previous data establish the use of the AFMU/1X ratio to discriminate fast from slow acetylators[1]; other data elegantly show that the AFMU/1X

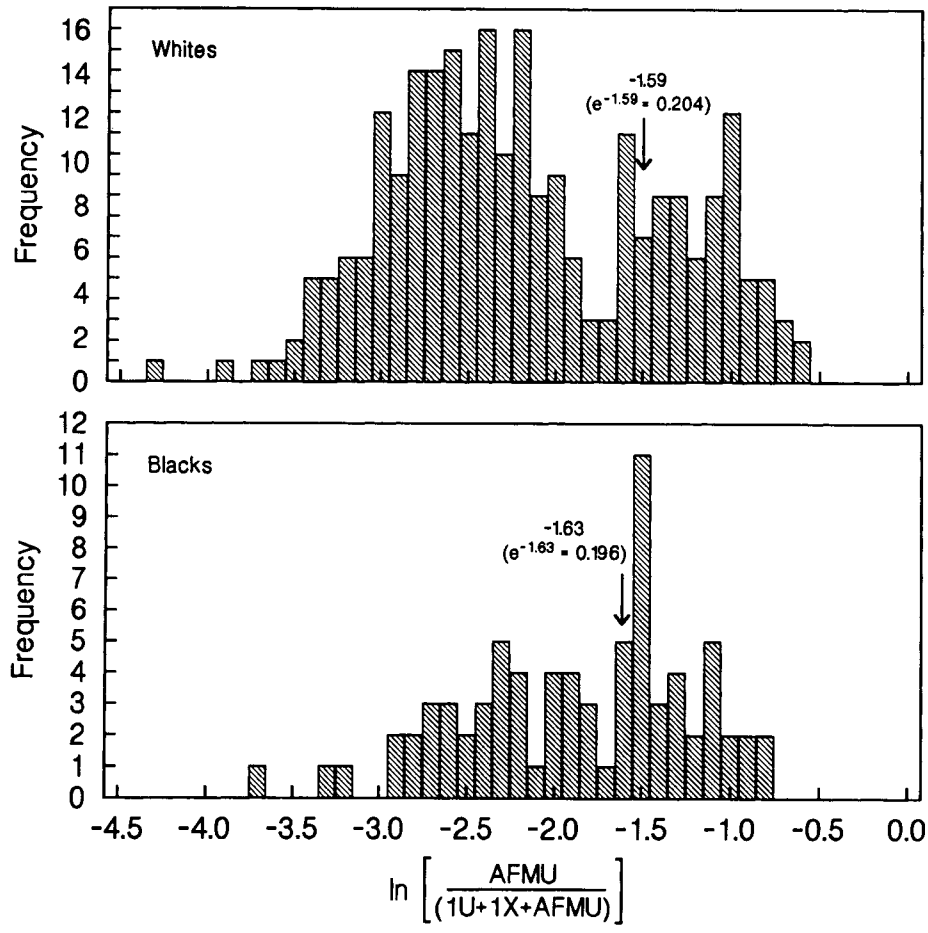

Fig. 4. Frequency distribution of log AFMU/(AFMU + 1U + 1X) in black subjects (n = 76) versus white subjects (n = 265), with maximum likelihood estimates of antimodes indicated.

ratio correlates well with in vitro sulfamethazine acetylation determined with liver biopsy cytosol from in vivo phenotyped subjects.[49] However, these previous data were generated in relatively small groups of white or Oriental subjects that did not include black subjects and in whom xanthine oxidase activity may have been less variable. As has been emphasized by others,[50] it is important to define the frequency distribution of metabolic ratios for a new test substrate in each individual ethnic group receiving the compound. Although we found that AFMU/1X ratios tended to be higher in black subjects than in white subjects, ANOVA revealed that gender was actually a stronger predictor of AFMU/1X (p = 0.03) than race (p = 0.08). This influence of gender, with females having higher AFMU/1X ratios, was somewhat surprising because others have not reported gender differences in NAT phenotypes.[11] However, the effect of gender on AFMU/1X was attributable to gender differences in xanthine oxidase activity and not to gender differences in NAT. Because we noted that females had a higher 1U/1X ratio than males, we hypothesized that the higher AFMU/1X ratios in females may be attributable to higher xanthine oxidase activity and thus lower 1X concentrations, which would then tend to elevate the AFMU/1X ratio, but not the other ratios used to assess NAT [AFMU/(1U + 1X) and AFMU/(AFMU + 1U + 1X)]. To test this, we constructed three separate linear regression models in which each of the three NAT indexes were the dependent and race, sex, and a xanthine oxidase index were the independent variables. Only the AFMU/1X ratio had as significant determinants both race *and* a xanthine oxidase index (log 1U/1X) (p = 0.003 and p = 0.001, respectively). For the other two NAT ratios, AFMU/(1U + 1X) and AFMU/(AFMU + 1U + 1X), only race was a signif-

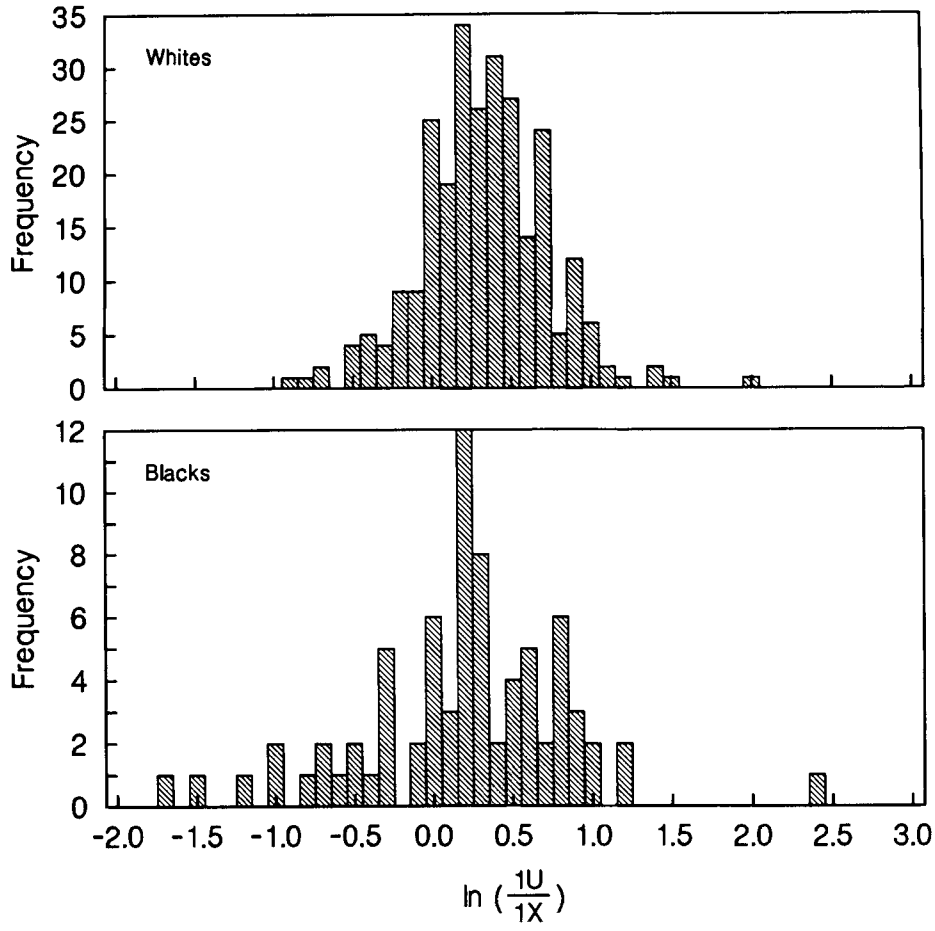

Fig. 5. Frequency distribution of log 1U/1X in black subjects (n = 75) versus white subjects (n = 265).

icant predictor at the 0.05 level (p = 0.002 and p = 0.001, respectively), with black subjects always having higher NAT ratios than white subjects.

In addition to the effects of gender, racial differences in xanthine oxidase activity also illustrate a limitation of the AFMU/1X ratio as an indicator of NAT activity in subgroup comparisons. The xanthine oxidase indexes were lower in black subjects than in white subjects. This fact helps to explain why, of the three NAT ratios, the AFMU/1X ratio exhibited the least discrimination in NAT activity between white subjects and black subjects. Xanthine oxidase activity was lower in black subjects, therefore they tended to have higher amounts of 1X, which in turn resulted in lower AFMU/1X ratios, which thus tended to obscure increased NAT activity in black subjects when AFMU/1X was used as the index of NAT. The alternative NAT indexes avoid the confounding effects of xanthine oxidase because they incorporate both 1U and 1X as nonacetylated alternative pathways for the PX intermediate.

NAT activity was therefore consistently higher in black subjects than in white subjects. However, the AFMU/(1U + 1X) and AFMU/(AFMU + 1U + 1X) ratios provide assessments of NAT activity less subject to the confounding variable of xanthine oxidase activity and may therefore provide more useful assessments of NAT activity when the study population includes both males and females and both black subjects and white subjects.

The higher NAT activity in black subjects may have relevance for their risk of cancers (e.g., bladder) associated with NAT activity,[19-21] and their responses to drugs (e.g., isoniazid and amonafide) in which acetylation phenotype has been associated with toxicity or efficacy.[11,16,18]

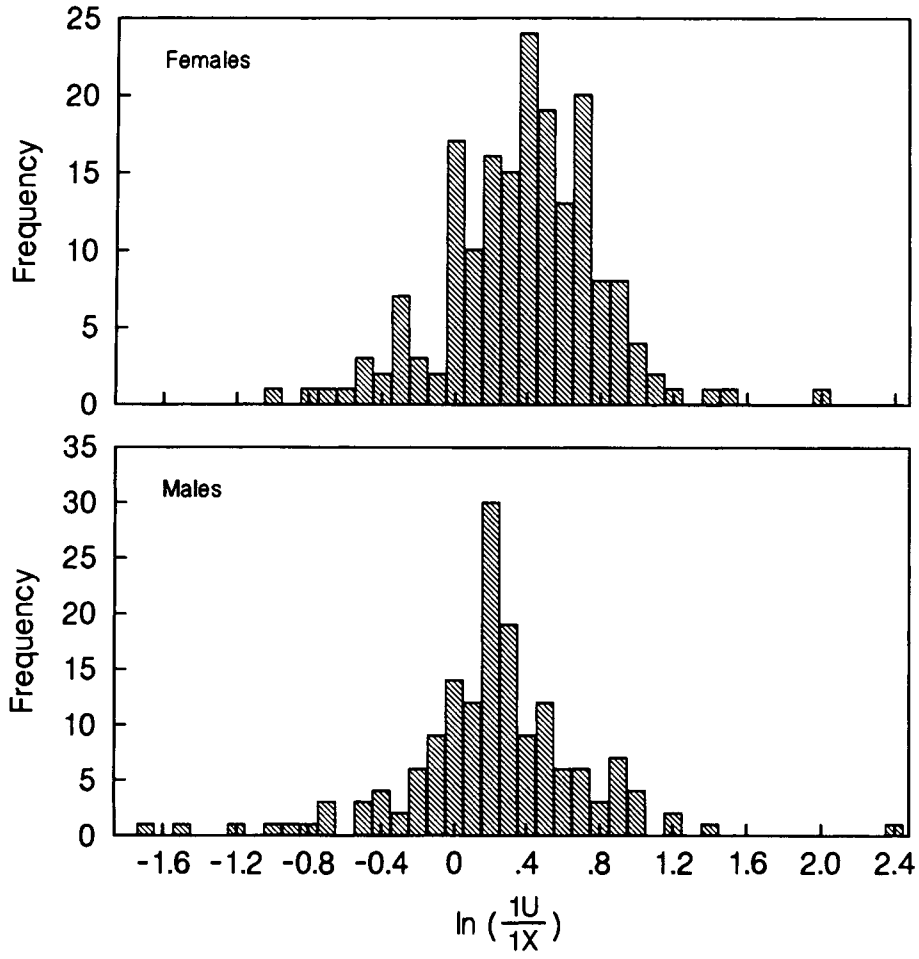

Fig. 6. Frequency distribution of log 1U/1X in males (n = 158) versus females (n = 182).

It should be acknowledged that most of the work that establishes caffeine metabolic ratios as an indicator of NAT phenotype has used AFMU/1X ratios to compare with independent assessments of phenotype (e.g., isoniazid acetylation).[1] Thus, although AFMU/(1U + 1X) and AFMU/(AFMU + 1U + 1X) are less subject to the influence of xanthine oxidase activity than AFMU/1X, they remain to be validated by comparison with NAT genotype or by comparison with NAT phenotype with use of another substrate. It should also be emphasized that the mathematic antimodes determined for the log AFMU/(1U + 1X) and log AFMU/(AFMU + 1U + 1X) ratios were based on the maximum likelihood estimates, assuming the data were drawn from a mixture of two normal distributions. Thus "phenotypes" assigned on the basis of these antimodes have not been confirmed genotypically or with family studies. Moreover, both AFMU/(1U + 1X) and AFMU/(AFMU + 1U + 1X) yielded rather low prevalences (i.e., <30%) of the fast acetylator phenotype in our white population, lower than most estimates of fast acetylator phenotype prevalences in white subjects.[11]

Whether 1U/1X or 1U/(1U + 1X) was used to assess xanthine oxidase, both race and gender were significant covariates, with black subjects and males having lower activity. This represents the first comparison of xanthine oxidase activity between white subjects and black subjects. The observed gender difference is in contrast to that previously reported,[40] where higher xanthine oxidase activity was found in liver biopsy cytosols from males than in those from females. Kalow and Tang[7] found no effects of gender on xanthine oxidase activity. The reasons for these discrepant findings on gender differences in xanthine oxidase activity are not clear. Likewise, the clinical importance of these racial and gender differences in xanthine oxidase activity is unknown at this point. As others have re-

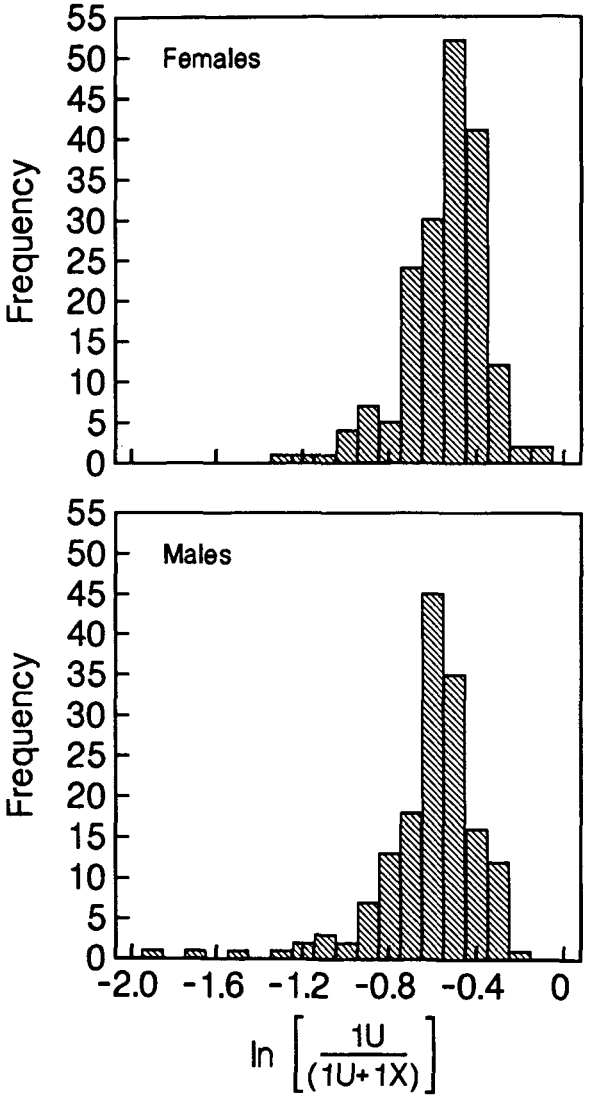

Fig. 7. Frequency distribution of log 1U/(1U + 1X) in males (n = 158) versus females (n = 182).

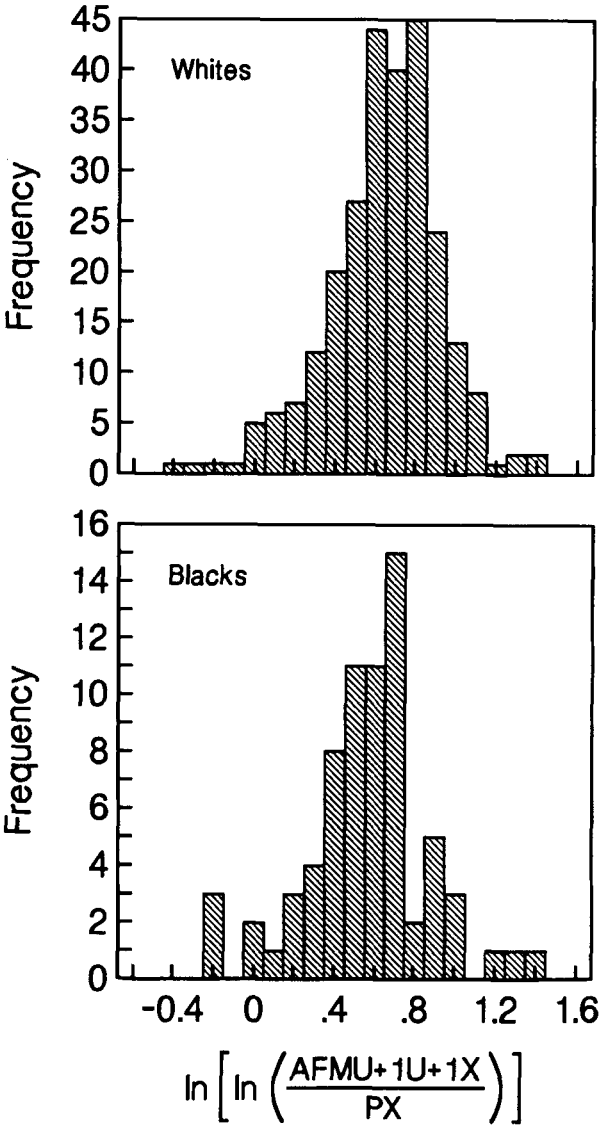

Fig. 8. Frequency distribution of log(log) (AFMU + 1U + 1X)/PX in black subjects (n = 71) versus white subjects (n = 260).

ported,[6] we found a few individuals (3 of 340, all of whom were black males) with very low xanthine oxidase indexes. We could discern nothing unusual about these three individuals.

Finally, we have used the ratio of (AFMU + 1U + 1X)/PX as a putative indicator of CYP1A2 activity. Similar to a previous report that used (AAMU + 1U + 1X)/1,7-DMU ratio[7] and one that used $^{13}CO_2$ exhalation after caffeine,[43] we found lower CYP1A2 ratios in females, despite the fact that none of our females were taking concurrent oral contraceptives. Race was also a significant predictor of the CYP1A2 ratio, with white subjects having higher activity (p = 0.036). These data may indicate a greater exposure of white subjects and males to polycyclic aromatic hydrocarbon-inducing agents or, alternatively, increased inducibility of CYP1A1 in white subjects and males. Whether these differences in CYP1A2 activity have any clinical significance in indicating either greater or reduced ability to metabolize carcinogenic hydrocarbons remains to be shown.

Admittedly, the best caffeine metabolic ratio with which to indicate CYP1A2 activity is somewhat con-

troversial. Demethylation of PX in vitro with microsomes from a number of human livers has been shown to correlate highly (r = 0.94) with ethoxyresorufin *O*-deethylation activity,[51] which is reflective of CYP1A2-type P450 activity. However, the best indicator in vivo has yet to be defined. The inclusion of urinary concentrations of PX in caffeine CYP1A2 indexes has been criticized because of variability in its renal elimination and its presence as a minor caffeine metabolite.[6,25] As an alternative, the ratio of (AAMU + 1U + 1X)/1,7-DMU has been proposed as an index of CYP1A2.[6,7,51] However, it should be noted that PX recovery constitutes about as large a percentage of a caffeine dose as 1,7-DMU[8] and other metabolites (e.g., AFMU) shown to be useful in assessing metabolizing enzymes.[8] In addition, PX demethylation has been found to correlate highly (r = 0.87) with PX oxidation to its uric acid derivative,[51] and thus inclusion of PX in the denominator of this ratio may provide as good as or a better index than ratios excluding it. Moreover, the range of (AFMU + 1U + 1X)/PX ratios that we observed comprises a very similar range to that reported with use of 1,7-DMU in the denominator.[6]

The correlations between metabolite concentrations are high (i.e., all with $r > 0.66$), probably because of the large range of overall concentration of urine samples. There are a few interesting observations from this correlation analysis. One is the relatively high correlation between xanthine oxidase activity ratios and AFMU/1X ($r > 0.3$), consistent with our findings that xanthine oxidase activity (i.e., 1U/1X ratio) had a significant effect on AFMU/1X in a regression model. Second, those ratios assessing the same enzyme activity (the three NAT ratios and the two xanthine oxidase ratios) are highly correlated with each other (all with $r > 0.9$). Third, it is encouraging that where one expects independence of enzyme activities (e.g., NAT or xanthine oxidase and CYP1A2 ratios), there are indeed very poor correlations (all with $r < 0.1$). This is in agreement with a report in which PX/caffeine ratios were increased in smokers while 1U/1X ratios were unaffected,[46] and it is in contrast to an earlier report[6] in which xanthine oxidase and CYP1A2 indexes were unaccountably significantly correlated ($r = -0.29$).

To assess whether differences in ratios between genders or between races were attributable primarily to differences in recovery of one metabolite over another, we compared the metabolite concentration data in males versus females and black subjects versus white subjects by use of ANOVA. Because no attempt

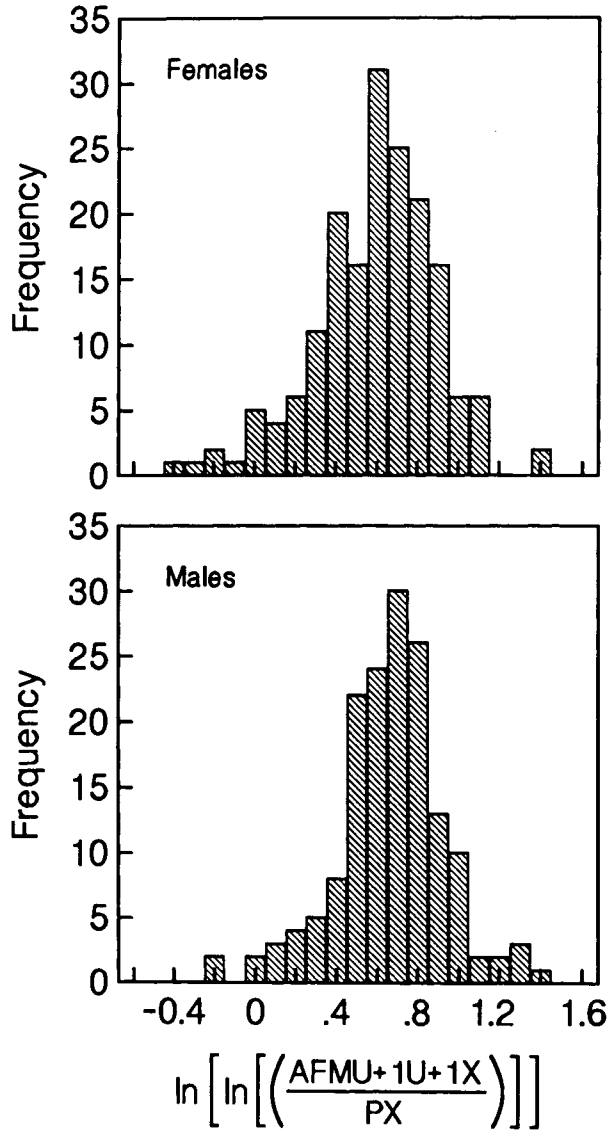

Fig. 9. Frequency distribution of log(log) (AFMU + 1U + 1X)/PX in males (n = 157) versus females (n = 174).

was made to standardize the fluid status or the volume of urine collection for each sample, we did not expect to find any racial or gender differences in the raw concentration data. However, it has been suggested that racial differences in the renal excretion of PX, as assessed by urinary concentrations, may account for racial differences in CYP1A2 ratios between white subjects and Oriental subjects.[25] In contrast, we observed no racial differences in the measured concentrations of any metabolite, which suggests that it is not altered renal excretion or urine stability of one metabolite or an-

other that could account for the racial differences in enzyme indexes that we observed (Table II). Lack of racial effects on renal excretion is also supported by the fact that there was no racial difference in the total excretion of all four metabolites. However, males had higher concentrations of all four caffeine metabolites than females. However, all four metabolites were affected, which indicates only that either males had greater nonrenal clearance of caffeine or that their urine samples tended to be more concentrated than females. This nonspecific gender difference in the degree of concentration of the urines does not account for the gender differences observed in the metabolic ratios based on these metabolite concentrations.

In this group of children and adults from 2 to 46 years of age, we found no relationships between age and any metabolic ratios. Children younger than 15 months of age were excluded because of the known alterations in caffeine metabolism that occur during infancy.[27] We did not observe decreases in the CYP1A2 index with increasing age over the age range encompassing puberty, as was observed with $^{13}CO_2$ exhalation after caffeine.[43] Perhaps this difference with $^{13}CO_2$ exhalation was reflective more of a change in overall caffeine clearance than with decreases in CYP1A2 activity. Age-related effects on enzyme indexes in infants and in adults older than 46 years of age cannot be excluded.

It should be acknowledged that the finding of differences in apparent NAT, xanthine oxidase, and CYP1A2 activities by race and gender do not necessarily imply genetically determined differences related to race or gender. Because environmental, occupational, and dietary histories were not defined, it is possible that the observed differences by race were caused primarily by different environmental exposures between, for example, white subjects and black subjects, and not to a different genetic constitution between the racial groups. Nonetheless, whatever the underlying mechanism, we have observed significant racial and gender differences in NAT and xanthine oxidase activities, as assessed with caffeine urinary metabolic ratios. Future studies will be needed to assess whether there is a genetic basis for these differences.

In summary, increased xanthine oxidase activity in females and in white subjects makes the AFMU/1X ratio less useful in assessing NAT activity than other caffeine NAT ratios in mixed populations. The increased NAT and decreased CYP1A2 and xanthine oxidase activities in black subjects and the decreased CYP1A2 and increased xanthine oxidase activity in females may have implications for racial and gender differences in drug and xenobiotic biotransformation. These factors should be considered in studies that use caffeine as a model substrate probe for drug metabolizing enzymes.

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