# Quantitation of three-month intraindividual variability and influence of sex and menstrual cycle phase on CYP1A2, *N*-acetyltransferase-2, and xanthine oxidase activity determined with caffeine phenotyping

*Objective:* To evaluate intraindividual variability and the effects of sex and menstrual cycle phase on the activity of cytochrome P450 1A2 (CYP1A2), *N*-acetyltransferase 2 (NAT2), and xanthine oxidase.
*Methods:* Ten white men were given 2 mg/kg caffeine orally every 14 days for 3 months. The same dosage of caffeine was given to 10 premenopausal white women during the midfollicular and midluteal phases of three complete menstrual cycles. Phenotype was determined with urinary caffeine metabolite ratios.
*Results:* For CYP1A2, mean metabolic ratio (±SD) was 5.97 ± 2.78 during the midfollicular phase and 5.32 ± 1.99 during the midluteal phase ($p = 0.2$). For extensive and poor metabolizers of NAT2, mean midfollicular phase metabolite ratios were 0.71 ± 0.060 and 0.37 ± 0.030, and mean midluteal phase metabolite ratios were 0.69 ± 0.076 and 0.39 ± 0.053 ($p = 0.9$). For xanthine oxidase, mean midfollicular phase metabolite ratio was 0.63 ± 0.06 and mean midluteal phase metabolite ratio was 0.63 ± 0.05 ($p = 0.3$). Among the men, mean CYP1A2, NAT2 rapid and slow acetylator, and xanthine oxidase indices were 9.42 ± 10.18, 0.66 ± 0.021, 0.31 ± 0.056, and 0.64 ± 0.03. There were no differences in metabolite ratios between men and women for CYP1A2, NAT2 extensive metabolizers, or xanthine oxidase. A statistically significant sex difference was found for poor metabolizers of NAT2 ($p < 0.05$). Median coefficients of variation for CYP1A2, NAT2 extensive and poor metabolizers, and xanthine oxidase ratios were 16.8% (range, 4.5% to 49.3%), 2.9% (range, 2.2% to 4.7%), 13.4% (range, 7.5% to 27.2%), and 4.5% (range, 2.3% to 13.0%).
*Conclusion:* Stratification by menstrual cycle phase or sex need not be performed for pharmacokinetic or clinical investigations of substrates for CYP1A2, NAT2, or xanthine oxidase in which the subjects are adults. (Clin Pharmacol Ther 1998;63:540-51.)

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Cytochrome P450 1A2 (CYP1A2) accounts for approximately 15% of the total P450 content of the liver. It is responsible for the metabolism of more than 30 drugs and the activation of environmental and chemical procarcinogens, including arylamines and polycyclic aromatic hydrocarbons.[1] A number of environ-

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mental and chemical compounds can induce and inhibit this enzyme.[2] Although marked interindividual differences in CYP1A2 activity have been found in homogeneous populations, it is currently unknown whether this enzyme is polymorphically expressed.[3-5]

*N*-Acetyltransferase-2 (NAT2) is a cytosolic enzyme present in liver and jejunal mucosa. It performs phase II conjugation reactions by detoxifying certain environmental carcinogens and acetylating more than 15 frequently used medications, such as isoniazid, hydralazine, procainamide, dapsone, and the sulfonamides.[6,7] NAT2 activity is polymorphic and exhibits ethnic and geographic variability,[8-10] which can have considerable influence on drug efficacy, toxicity,[11] and risk for specific neoplasms.[12,13]

Xanthine oxidase is a soluble cytoplasmic enzyme with highest activity in the liver and intestine.[14] It is responsible for the metabolism of endogenous compounds (purines and pyrimidines) and medications such as mercaptopurine and azathioprine. The activity of xanthine oxidase is not polymorphic, but twofold to fourfold ranges in activity have been found among adults.[15,16] Little is known about the role of xanthine oxidase variability as it relates to differences in drug metabolism.

Although much work has been done in characterizing interindividual variability of CYP1A2, NAT2, and xanthine oxidase activity, no studies to date have examined long-term intraindividual variability of activity as determined with phenotyping. It is important to establish baseline variability in metabolic ratios used for phenotyping. This step determines the degree of change in enzyme activity that should be considered clinically significant for an individual. Phenotyping of these enzymes can be performed by administering a single oral dose of caffeine, a ubiquitous and frequently ingested compound. Quantitation of caffeine metabolites in a timed urine collection can be used to provide simultaneous estimates of the activities of the enzymes CYP1A2, NAT2, and xanthine oxidase.[17-19]

Because the U.S. Food and Drug Administration encourages inclusion of women in clinical trials,[20] it is important to determine whether sex or menstrual cycle phase affects the activity of drug-metabolizing enzymes. Some authors have suggested that normal hormonal changes during the menstrual cycle may contribute to differences in the pharmacokinetics and pharmacodynamics of drugs.[21,22] The limited available data are difficult to interpret, because many investigations have not been consistent in the menstrual cycle day studied or have used pharmacokinetic approaches with pharmacologic substrates, the metabolism of which depends on several different enzymes.

The literature addressing the effects of sex and menstrual cycle on CYP1A2 activity is conflicting. Limited data suggest that lower CYP1A2 activity occurs among women.[3-5,23,24] However, these differences may have been caused by the effects of parity or use of oral contraceptive drugs. One study showed lower CYP1A2 activity among Chinese women but not among a white population.[25] Investigations of sex differences in CYP1A2 activity generally have not controlled for menstrual cycle phase. Investigations of menstrual cycle effects on the metabolism of CYP1A2 substrates, such as caffeine, theophylline, methaqualone, and acetaminophen (INN, paracetamol), have shown shorter half-lives during the middle phase of the menstrual cycle and slower clearances during the luteal phase.[21,26-28]

With both dapsone and caffeine as probes,[23,29-31] no effect of sex on the distribution of NAT2 phenotype has been reported. There have been no investigations of the effects of menstrual cycle on NAT2 activity. Only two reports of sex differences in xanthine oxidase activity exist. One in vitro investigation found higher xanthine oxidase activity in the hepatic tissue of men,[16] and one in vivo investigation in which caffeine was used as a phenotyping probe showed lower xanthine oxidase activity among men.[23] To date there have been no investigations of the effects of menstrual cycle on xanthine oxidase activity.

To address these discrepancies, we conducted a study with the following specific aims: (1) to determine whether the activities of CYP1A2, NAT2, and xanthine oxidase as assessed with urinary metabolite ratios for caffeine differ during the midfollicular and midluteal phases of the menstrual cycle, (2) to determine whether the activities of CYP1A2, NAT2, and xanthine oxidase differ between men and women, and (3) to characterize intraindividual variability of CYP1A2, NAT2, and xanthine oxidase activity over 12 weeks among men and premenopausal women.

## METHODS

This study was approved by the institutional review board of Bassett Healthcare (Cooperstown, N.Y.), and written informed consent was obtained from all subjects.

***Study subjects.*** Using data from Tang et al.[32] with a two-sided $\alpha = 0.05$ and a two-sided $\beta = 0.80$, we estimated that a sample size of nine was needed to detect a 50% difference in caffeine metabolite ratios.[33] To allow for stratification by sex, 10 men and 10 premenopausal women (total of 20 subjects) were recruited for this investigation.

**Table I.** Demographic data for subjects

| *Variable* | *Men (n = 10)* | *Women (n = 10)* |
|---|---|---|
| Age (yr) | 34.8 ± 7.9 | 38.2 ± 9.3 |
| Total body weight (kg) | 76.3 ± 13.3 | 79.4 ± 20.1 |
| Ideal body weight (kg)* | 74.0 ± 6.8 | 58.5 ± 6.3† |
| AST (U/L) | 24.2 ± 4.8 | 24.0 ± 7.4 |
| ALT (U/L) | 30.1 ± 8.3 | 24.6 ± 9.2 |
| Serum creatinine (mg/dl) | 0.98 ± 0.08 | 0.82 ± 0.1† |
| Serum creatinine (ml/min/1.73 $m^2$)‡ | 99.4 ± 14.7 | 81.3 ± 19.7 |
| Caffeine dose (mg) | 155 ± 43.8 | 150 ± 33.3 |
| Length of overnight urine collection (hr) | 15.0 ± 1.0 | 14.3 ± 0.6 |
| Length of menstrual cycle (days) | — | 27.2 ± 2.3 |
| Follicular phase study day (days after menstruation) | — | 5.6 ± 1.4 |
| Menstrual cycle day of ovulation | — | 14.1 ± 2.8 |
| Luteal phase study day (days after menstruation) | — | 18.3 ± 3.3 |
| Luteal phase study day (days after menstruation) | — | 3.9 ± 1.3 |

Data expressed as mean values ± SD.
*See Devine.[38]
†$p < 0.05$ compared with men.
‡See Cockcroft and Gault.[39]

**Table II.** Menstrual cycle phase caffeine urinary metabolic ratios for CYP1A2, NAT2, and xanthine oxidase phenotyping

| | *CYP1A2* | | *NAT2* | | *XO* | |
|---|---|---|---|---|---|---|
| *Subject No.* | *Follicular* | *Luteal** | *Follicular†* | *Luteal†* | *Follicular* | *Luteal‡* |
| 1 | 3.90 ± 0.57 | 3.52 ± 0.19 | 0.40 ± 0.026 | 0.40 ± 0.081 | 0.62 ± 0.023 | 0.60 ± 0.057 |
| 2 | 7.34 ± 0.72 | 8.48 ± 2.04 | 0.39 ± 0.0075 | 0.42 ± 0.049 | 0.64 ± 0.0085 | 0.64 ± 0.057 |
| 3 | 4.55 ± 0.27 | 4.64 ± 0.56 | 0.37 ± 0.033 | 0.34 ± 0.058 | 0.64 ± 0.047 | 0.58 ± 0.033 |
| 4 | 3.16 ± 0.98 | 3.55 ± 0.68 | 0.37 ± 0.11 | 0.48 ± 0.076 | 0.54 ± 0.094 | 0.62 ± 0.034 |
| 5 | 5.00 ± 0.62 | 4.97 ± 0.55 | 0.64 ± 0.019 | 0.61 ± 0.032 | 0.72 ± 0.019 | 0.71 ± 0.038 |
| 6 | 5.20 ± 2.52 | 3.68 ± 0.57 | 0.36 ± 0.024 | 0.34 ± 0.067 | 0.65 ± 0.020 | 0.64 ± 0.015 |
| 7 | 12.80 ± 6.39 | 8.18 ± 3.14 | 0.39 ± 0.037 | 0.38 ± 0.035 | 0.56 ± 0.071 | 0.56 ± 0.028 |
| 8 | 7.17 ± 0.70 | 6.98 ± 0.77 | 0.72 ± 0.014 | 0.70 ± 0.023 | 0.71 ± 0.022 | 0.70 ± 0.030 |
| 9 | 6.50 ± 0.68 | 6.00 ± 0.73 | 0.79 ± 0.014 | 0.76 ± 0.025 | 0.59 ± 0.039 | 0.60 ± 0.0048 |
| 10 | 4.12 ± 0.47 | 3.17 ± 0.90 | 0.31 ± 0.10 | 0.35 ± 0.012 | 0.63 ± 0.0099 | 0.63 ± 0.020 |
| All women | 5.97 ± 2.78 | 5.32 ± 1.99<br>0.37 ± 0.030‖ | 0.70 ± 0.060§<br>0.39 ± 0.053‖ | 0.69 ± 0.076§ | 0.63 ± 0.057 | 0.63 ± 0.048 |

Data expressed as mean values ± SD.
Follicular, Mean of three midfollicular phase metabolic ratios; luteal, mean of three midluteal phase metabolic ratios.
*$p = 0.20$ by Wilcoxon signed-rank test versus midfollicular ratio.
†$p = 0.88$ by Wilcoxon signed-rank test versus midfollicular ratio for both extensive and poor metabolizers.
‡$p = 0.28$ by Wilcoxon signed-rank test versus midfollicular ratio.
§Extensive metabolizers.
‖Poor metabolizers.

A thorough history was obtained from all subjects and each subject had a physical examination, electrocardiogram, blood chemistry, and urinalysis screening before the study. Subjects were excluded if they were receiving any medications on an ongoing basis or were receiving concomitant therapy with drugs known to induce or inhibit the cytochromes P450. Smokers and binge drinkers also were excluded. Moderate alcohol intake was allowed (one drink equivalent to one 12-ounce beer daily). Subjects were excluded if their hepatic transaminase levels (AST and ALT) were more than 1.5 times the upper limit of normal (AST from 0 to 50 U/L and ALT from 0 to 60 U/L for men; AST from 0 to 40 U/L and ALT from 0 to 50 U/L for women), if their bilirubin level was greater than 1.5 mg/dl, or if their serum creatinine was not within the normal range (0.6 to 1.2 mg/dl for men; 0.5 to 1.0 mg/dl for women). Women were required to have uniform menstrual cycles, defined as a predictable cycle length (i.e., ±3 days) in a 3-month his-

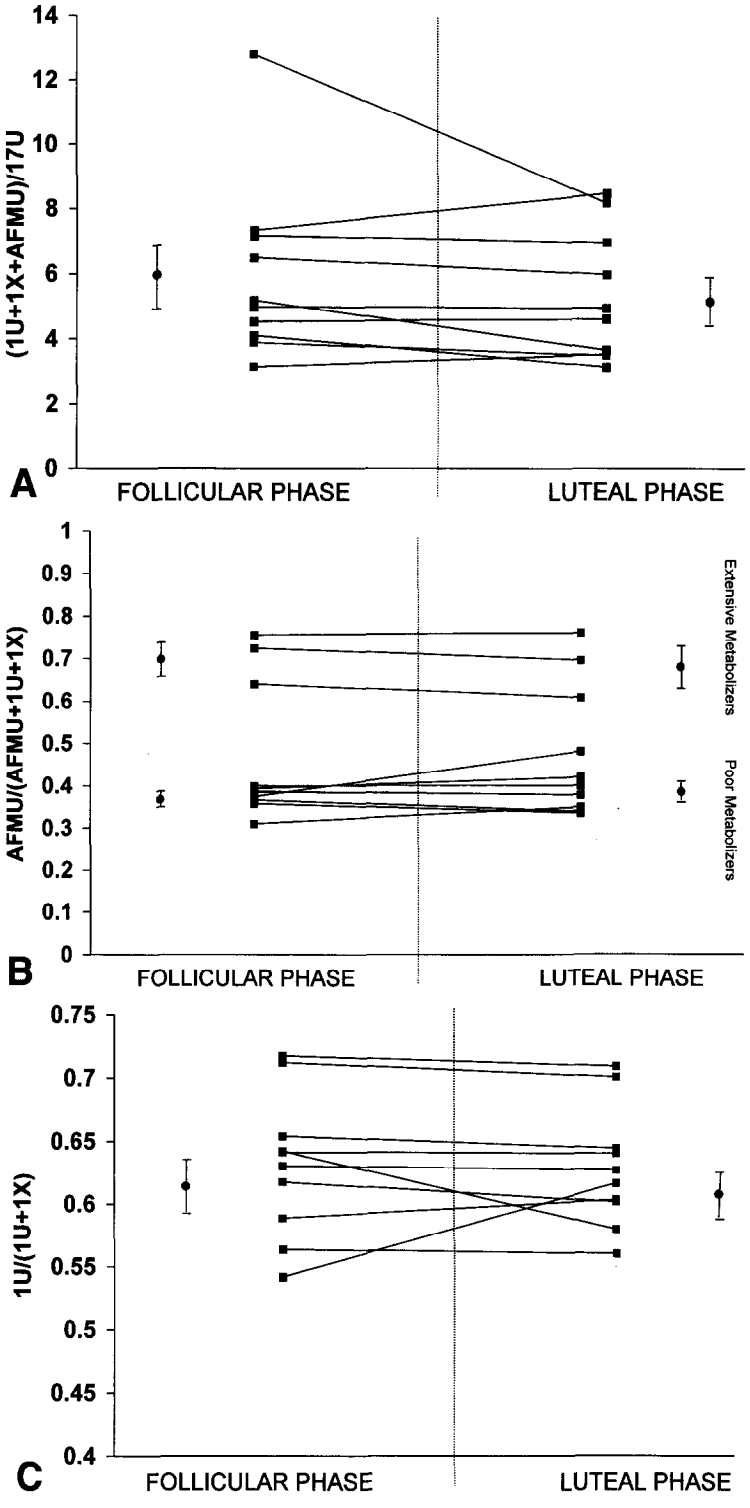


**Fig. 1.** Mean urinary caffeine metabolic ratios for phenotyping drug-metabolizing enzymes during the midfollicular and midluteal phases of the menstrual cycle for 10 women. Data are individual subject and combined mean values ± SEM. **A,** Mean urinary caffeine metabolic ratios for phenotyping CYP1A2. **B,** Mean urinary caffeine metabolic ratios for phenotyping NAT2. **C,** Mean urinary caffeine metabolic ratios for phenotyping xanthine oxidase. 1U, 1-Methyluric acid; 1X, 1-methylxanthine; AFMU, 5-acetylamino-6-formylamino-3-methyluracil; 17U, 1,7-dimethyluric acid.

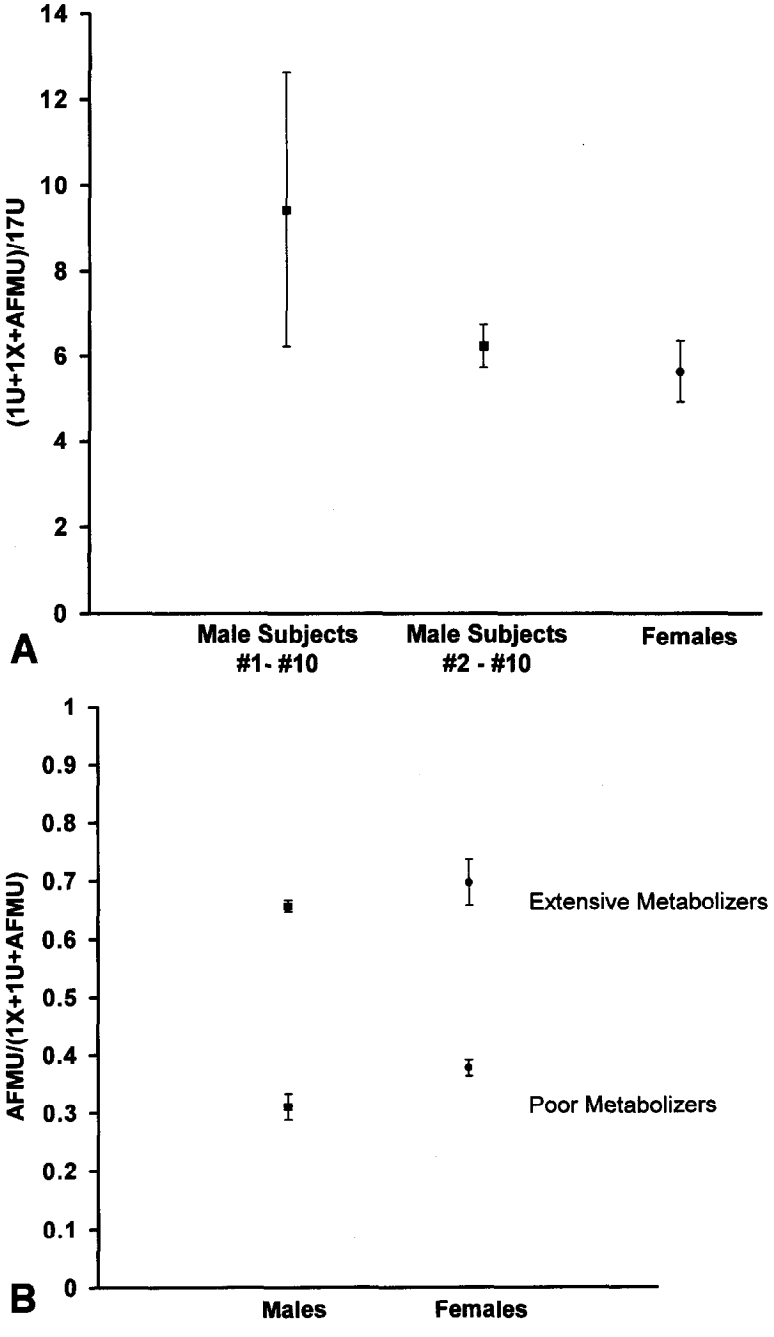


**Fig. 2.** Comparison of mean ± SEM urinary caffeine metabolic ratios for men and women. **A,** Comparison of CYP1A2 metabolic ratios for 10 men, 9 men (subject 1 was excluded), and 10 women. **B,** Comparison of NAT2 metabolic ratios for 10 men and 10 women. **C,** Comparison of xanthine oxidase metabolic ratios for 10 men and 10 women.

tory. Women who were not surgically sterile underwent urine pregnancy testing (qualitative β–human chorionic gonadotropin) during screening and before each study phase (One-Step Clearblue Easy; Whitehall Laboratories, Madison, N.J.).

***Phenotyping procedure.*** Male subjects were phenotyped once every 14 days for 12 weeks. Women were phenotyped during the midfollicular and midluteal phases of their menstrual cycle for three complete cycles. To establish menstrual cycle patterns, women

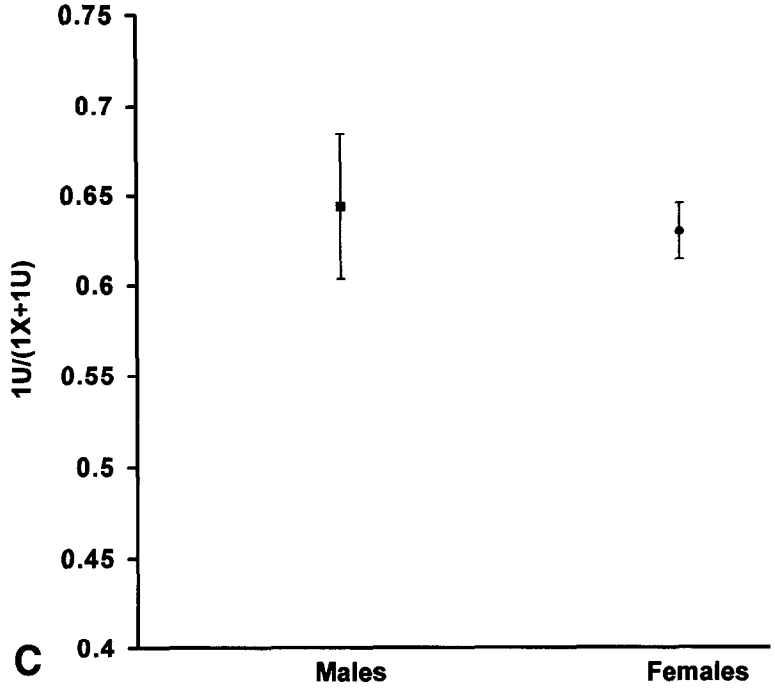


**Fig. 2.** *(cont'd)* Comparison of mean ± SEM urinary caffeine metabolic ratios for men and women. **A,** Comparison of CYP1A2 metabolic ratios for 10 men, 9 men (subject 1 was excluded), and 10 women. **B,** Comparison of NAT2 metabolic ratios for 10 men and 10 women. **C,** Comparison of xanthine oxidase metabolic ratios for 10 men and 10 women.

were instructed to keep a diary for 3 months before beginning the study and to use a home diagnostic ovulation kit for qualitative luteinizing hormone (One-Step Clearplan Easy; Whitehall Laboratories) during the menstrual cycle before the beginning of the study. During each month of study, female subjects were instructed to test first morning urine for qualitative luteinizing hormone 3 days before predicted midcycle and to continue until a positive result was found to accurately determine ovulation. Women were phenotyped during the midfollicular (days 5 through 8 of the cycle) and midluteal (days 17 through 20 of the cycle) phases of the menstrual cycle. All subjects refrained from ingesting ethanol, chocolate, caffeine-containing beverages, grapefruit or grapefruit juice, charbroiled foods, watercress, and cruciferous vegetables (e.g., broccoli, cauliflower, cabbage, brussels sprouts, and kale) for 3 days before and for the day of each phenotyping study.

At 4 PM, subjects emptied their bladders and were given an oral dose of 2 mg caffeine per kilogram of total body weight, rounded to the nearest 50 mg (NoDoze; Bristol Myers, Princeton, N.J.) and an oral dose of 30 mg dextromethorphan (Robitussin Pediatric Solution; AH Robbins, Madison, N.J.) for concurrent evaluation of CYP2D6 activity. All urine was collected overnight in a single container with 2 gm ascorbic acid, up to and including the first morning void. Total time of urine collection, total urine volume, and urine pH were recorded after mixing of the specimen. Fifteen-milliliter aliquots of urine were combined with 20 mg ascorbic acid per milliliter of urine to maintain pH less than 4 and were frozen at –80° C until analysis.

***Analytical procedure.*** Determination of 1-methyluric acid (1U), 1-methylxanthine (1X), 1,7-dimethyluric acid (17U), and 5-acetylamino-6-formylamino-3-methyluracil (AFMU) in urine was accomplished with a modification of the HPLC methods of Evans et al.[34] The internal standard (sulfamethoxazole) was added to 400 μl urine saturated with approximately 2420 mg ammonium sulfate. The caffeine metabolites were then extracted with 3.5 ml solution of chloroform/2-propanol (90:10). The aqueous layer was removed by means of aspiration and discarded. The organic layer was dried (SpeedVac; Savant, Holbrook, N.Y.) at 50° C. Samples were dissolved in 100 μl of 0.05% acetic acid, and 75 μl was injected into the HPLC system (HP model 1100 chromatographic system; Hewlett-Packard,

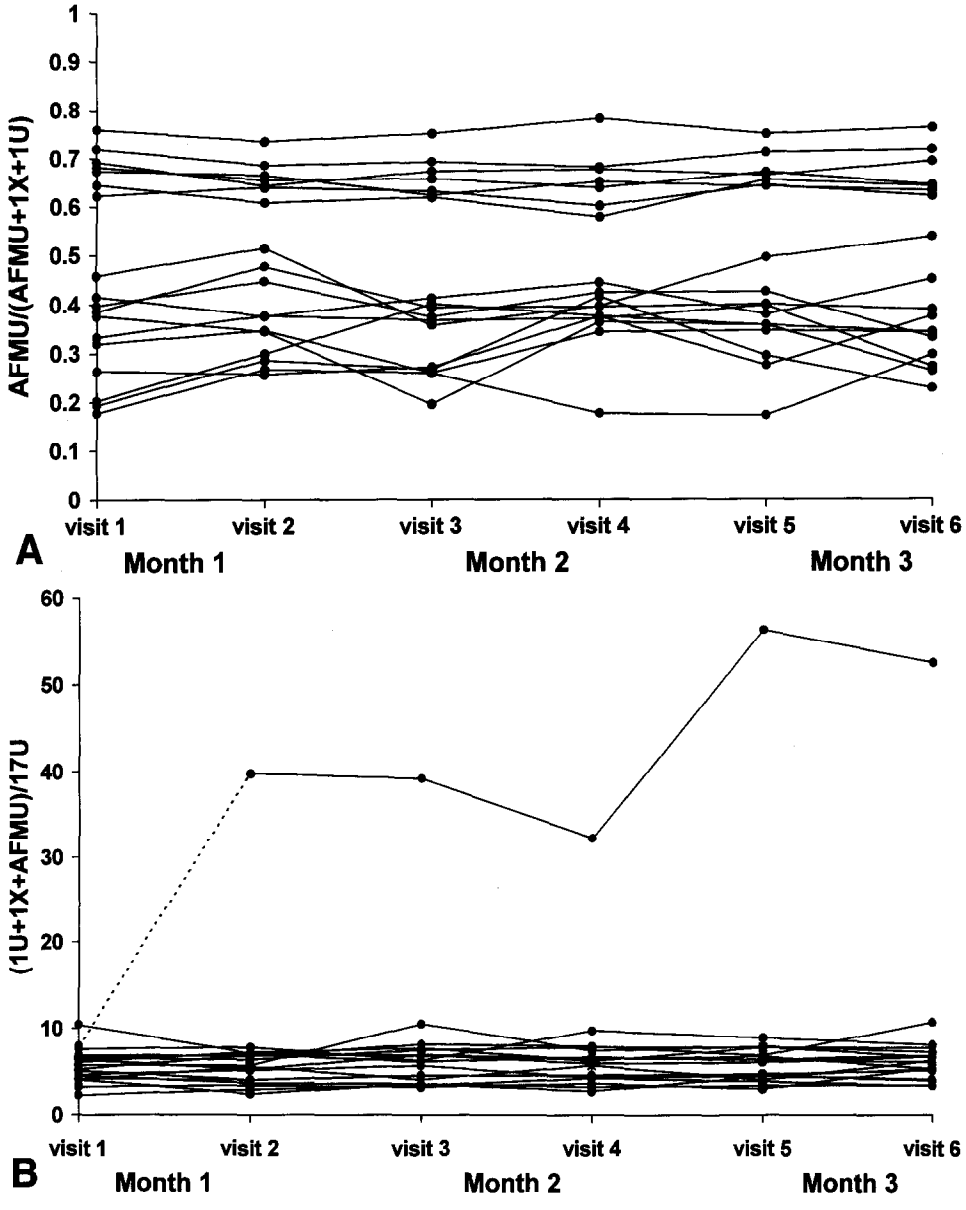


**Fig. 3.** Sequential drug-metabolizing enzyme activity measured with caffeine urinary metabolic ratios for eight women during three consecutive menstrual cycles and 10 men over 3 months. **A,** Sequential CYP1A2 activity. **B,** Sequential NAT2 activity. **C,** Sequential xanthine oxidase activity.

San Fernando, Calif.). Chromatography was performed at 50° C on a Nova-Pak $C_{18}$ column (Waters, Milford, Mass.) equilibrated with 95% solvent A (0.05% acetic acid) and 5% solvent B (100% methanol) at a flow rate of 1.2 ml/min. The chromatogram was developed with a multistep gradient produced by means of applying the following linear change in solvent: 0 minutes, 5%; 3.0 minutes, 5%; 5.0 minutes, 7%; 6.0 minutes, 15%; 7.0 minutes, 20%; 10.01 minutes, 30%; 14.0 minutes, 35%; 14.01 minutes, 100%; 17 minutes, 100%; 17.01 minutes, 5%. Ultraviolet detection at 290 nm was used to monitor the separation of analytes. Data output was normalized to the internal standard, and the molar amount was determined with standard curves prepared daily in drug-free urine specimens.

Intraday variability for 17U at concentrations of 500, 100, 10, and 1 nm/ml was 4.9%, 5.5%, 3.3%, and 10.5%; interday variability was 4.3%, 8.4%, 8.0%, and 14.2%. Intraday variability for 500, 100, 10, and 1 nm/ml concentrations of 1X was 5.5%, 2.0%, 5.9%,

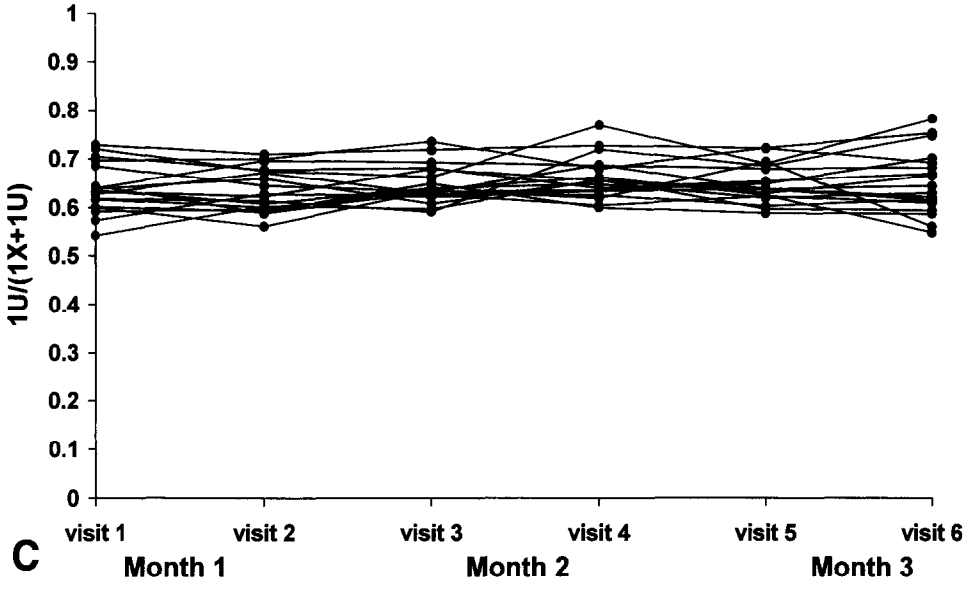


**Fig. 3.** *(cont'd)* Sequential drug-metabolizing enzyme activity measured with caffeine urinary metabolic ratios for eight women during three consecutive menstrual cycles and 10 men over 3 months. **A,** Sequential CYP1A2 activity. **B,** Sequential NAT2 activity. **C,** Sequential xanthine oxidase activity.

and 4.6%; interday variability was 10.5%, 9.3%, 6.2%, and 21.0%. For 1U, the intraday and interday variability were consistently less than 10%. Intraday variability for AFMU at concentrations of 100, 10, and 1 nm/ml was 5.2%, 4.1%, and 4.0%; interday variability was 8.3%, 5.2%, and 4.0%.

***Material.*** AFMU was obtained from Dr. B. K. Tang at the Department of Pharmacology, University of Toronto, Ontario, Canada. All other standards and reagents used for quantitation of caffeine metabolites were of the highest purity available commercially (Sigma Chemical Co., St. Louis, Mo.).

***Phenotype assignment.*** Demethylation ratios were used to express CYP1A2 activity.[17,35] The CYP1A2 index was defined as molar ratio of (1U+1X+AFMU)/17U. Hydroxylation molar ratios were used to express xanthine oxidase activity as follows: 1U/1X and 1U/(1U+1X).[19] Three molar ratios for NAT2 were examined, as follows: AFMU/1X, AFMU/(1X+1U), and AFMU/(1X+1U+AFMU).[36]

***Statistical analysis.*** Statistical analysis was performed with SYSTAT software (version 5.02).[37] Normal distribution was determined with tests of skewness and kurtosis. The Wilcoxon signed-rank test was used to determine statistical significance of metabolic ratio variations between menstrual cycle phases. The Mann-Whitney rank sum test was used to determine statistical significance of metabolic ratios between men and women. Data are presented as mean values ± SD unless otherwise indicated. The significance limit accepted for all statistical analyses was $\alpha = 0.05$.

## RESULTS

All subjects completed all six phenotyping visits. Within the three consecutive menstrual cycles, one woman missed a midfollicular phenotyping visit and one woman missed a midluteal phenotyping visit. These two women had to continue into a fourth month to complete their data sets.

Subject demographic data are presented in Table I. Statistically significant differences existed between men and women with respect to ideal body weight and serum creatinine concentration. There were no other significant differences between these two groups. Of the two xanthine oxidase and three NAT2 metabolic indices, 1U/(1U+1X) ratio for xanthine oxidase and AFMU/(1U+1X+AFMU) ratio for NAT2 resulted in the least amount of variability. Although only these ratios are reported, statistical analyses were performed on all NAT2 indices that produced similar results to those for the aforementioned metabolite ratios (data not shown).

***Influence of menstrual cycle phase on CYP1A2, NAT2, and xanthine oxidase activity.*** The mean values for the three midfollicular and midluteal caffeine metabolic ratio measurements for each female subject

**Table III.** Caffeine urinary metabolic ratios and coefficients of variation for CYP1A2, NAT2, and xanthine oxidase phenotyping of women and men

| | *CYP1A2* | | *NAT2* | | *Xanthine oxidase* | |
|---|---|---|---|---|---|---|
| *Subject No.* | *Metabolic ratio** | *Coefficient of variation (%)* | *Metabolic ratio†* | *Coefficient of variation (%)* | *Metabolic ratio‡* | *Coefficient of variation (%)* |
| *Women* | | | | | | |
| 1 | 3.71 ± 0.44 | 11.74 | 0.40 ± 0.042 | 10.45 | 0.61 ± 0.040 | 6.51 |
| 2 | 7.91 ± 1.51 | 19.03 | 0.41 ± 0.035 | 8.57 | 0.64 ± 0.037 | 5.70 |
| 3 | 4.59 ± 0.40 | 8.66 | 0.35 ± 0.045 | 12.6 | 0.61 ± 0.050 | 8.18 |
| 4 | 3.36 ± 0.78 | 23.3 | 0.43 ± 0.11 | 24.5 | 0.58 ± 0.076 | 13.05 |
| 5 | 5.00 ± 0.52 | 10.51 | 0.62 ± 0.029 | 4.66 | 0.71 ± 0.027 | 3.83 |
| 6 | 4.44 ± 1.83 | 41.2 | 0.35 ± 0.046 | 13.4 | 0.65 ± 0.017 | 2.61 |
| 7 | 10.5 ± 5.17 | 49.3 | 0.38 ± 0.033 | 8.52 | 0.56 ± 0.048 | 8.59 |
| 8 | 7.08 ± 0.67 | 9.46 | 0.71 ± 0.023 | 3.19 | 0.71 ± 0.024 | 3.42 |
| 9 | 6.25 ± 0.69 | 11.01 | 0.76 ± 0.018 | 2.40 | 0.60 ± 0.026 | 4.42 |
| 10 | 3.64 ± 0.83 | 22.70 | 0.33 ± 0.067 | 20.29 | 0.63 ± 0.014 | 2.29 |
| All women | 5.65 ± 2.28 | 20.69 ± 14.15 | 0.70 ± 0.067§<br>0.38 ± 0.036‖ | 3.42 ± 1.15§<br>14.06 ± 6.12‖ | 0.63 ± 0.050 | 5.86 ± 3.34 |
| *Men* | | | | | | |
| 1 | 38.12 ± 17.26 | 45.30 | 0.66 ± 0.014 | 2.15 | 0.63 ± 0.051 | 8.12 |
| 2 | 7.26 ± 1.59 | 21.94 | 0.33 ± 0.034 | 10.43 | 0.62 ± 0.023 | 3.69 |
| 3 | 4.40 ± 0.46 | 10.42 | 0.40 ± 0.030 | 7.50 | 0.62 ± 0.039 | 6.25 |
| 4 | 6.30 ± 0.77 | 12.15 | 0.67 ± 0.019 | 2.86 | 0.65 ± 0.025 | 3.80 |
| 5 | 7.16 ± 0.78 | 10.88 | 0.63 ± 0.015 | 2.47 | 0.68 ± 0.020 | 2.93 |
| 6 | 5.00 ± 1.00 | 20.05 | 0.30 ± 0.056 | 18.54 | 0.63 ± 0.023 | 3.69 |
| 7 | 8.09 ± 1.18 | 14.62 | 0.28 ± 0.077 | 27.23 | 0.66 ± 0.064 | 9.67 |
| 8 | 3.77 ± 0.85 | 22.56 | 0.23 ± 0.050 | 21.32 | 0.71 ± 0.059 | 8.41 |
| 9 | 6.63 ± 0.30 | 4.53 | 0.67 ± 0.023 | 3.44 | 0.65 ± 0.019 | 2.93 |
| 10 | 7.52 ± 1.75 | 23.3 | 0.32 ± 0.072 | 22.49 | 0.60 ± 0.028 | 4.64 |
| All men | 9.42 ± 10.18 | 18.58 ± 11.28 | 0.66 ± 0.021§<br>0.31 ± 0.056‖ | 2.73 ± 0.56§<br>17.92 ± 7.54‖ | 0.64 ± 0.032 | 5.41 ± 2.51 |
| Median for all men¶ | 6.89 (5.33-7.46) | 17.34 (11.2-22.4) | | | | |

All data expressed as mean value ± SD. Metabolic ratio is mean value of six metabolic ratio measures over a 3-month period.
*$p = 0.16$ by Mann-Whitney rank sum test versus male CYP1A2 ratio.
†$p = 0.33$ by Mann-Whitney rank sum test versus male NAT2 ratio for extensive metabolizers, and $p < 0.05$ for poor metabolizers.
‡$p = 0.31$ by Mann-Whitney rank sum test versus male xanthine oxidase ratio.
§Extensive metabolizers.
‖Poor metabolizers.
¶25th to 75th percentile given in parentheses.

are listed in Table II. Fig. 1 illustrates the mean values of the CYP1A2, NAT2 (extensive and poor metabolizers), and xanthine oxidase indices between the follicular and luteal phases of the menstrual cycle for each subject and the average midfollicular and midluteal phase indices for all 10 women. No trend between menstrual cycle phases was noted. No significant difference in metabolic ratios existed between the midfollicular and midluteal menstrual cycle phases for CYP1A2, NAT2 extensive and poor metabolizers, or xanthine oxidase ($p = 0.2$, $p = 0.8$, $p = 0.7$, and $p = 0.3$, respectively). No significant differences were found in variability between CYP1A2, NAT2 extensive and poor metabolizers, or xanthine oxidase indices for the midfollicular and midluteal phases (mean coefficient of variation values were 20.4% versus 17.8%, 8.6% versus 11.0%, 8.1% versus 11.5%, and 5.9% versus 5.1%, respectively) of the menstrual cycle. As a result, the midfollicular and midluteal phase metabolic ratios were combined for each subject, and the mean values were used in all subsequent analyses.

***Influence of sex on CYP1A2, NAT2, and xanthine oxidase activity.*** Mean caffeine metabolic ratios for all subjects are listed in Table III. Fig. 2 depicts the pooled caffeine metabolic ratios for men and women. No statistically significant difference existed between the mean metabolic ratios of men and women for CYP1A2, NAT2 extensive metabolizers (four men, three women),

or xanthine oxidase. A statistically significant difference did exist in caffeine metabolic ratios between the seven female and six male poor metabolizers of NAT2 (female ratios were 23% higher than male ratios; $p < 0.05$). However, when the indices for both NAT2 phenotypes were combined, no significant difference was found between men and women ($p = 0.33$).

***Intraindividual variability in caffeine metabolic ratios.*** Fig. 3 illustrates the caffeine metabolic ratios for eight women and 10 men who underwent consecutive phenotyping. The two female subjects who had data collected out of phase because they missed one of their consecutive midluteal or midfollicular phenotyping visits are not included in Fig. 3. However, the data for these subjects did not differ from those who had consecutive visits and thus were included in all statistical analyses. Fig. 2, *A*, illustrates data for a point noted for male subject 1 that appeared discrepant. No apparent explanation, such as changes in medication, diet, or illness, could be attributed to this apparent decrease in CYP1A2 activity, and repeated assay of the urine samples yielded similar metabolic ratios. As a result, statistical analyses of CYP1A2 differences between men and women were conducted both with and without data from this subject. Comparable results were seen regardless of inclusion or exclusion of these data.

Each subject's coefficients of variation for mean CYP1A2, NAT2, and xanthine oxidase indices are listed in Table III. No statistically significant differences were found between men and women for the variability in CYP1A2, NAT2, or xanthine oxidase indices ($p = 1.0$, $p = 0.9$, and $p = 0.9$, respectively). Coefficients of variation for NAT2 extensive metabolizers were significantly smaller than those for NAT2 poor metabolizers for both men and women ($p < 0.05$). For the CYP1A2 index, coefficients of variation ranged from 4.53% to 49.30%. For the NAT2 extensive and poor metabolizer indices, coefficients of variation ranged from 2.15% to 4.66% and 7.50% to 27.23%. For the xanthine oxidase index, coefficients of variation ranged from 2.29% to 13.05%.

## DISCUSSION

Drug-metabolizing enzyme activity may be influenced by hormonal fluctuations.[21,22] To test this hypothesis, we performed caffeine phenotyping for CYP1A2, NAT2, and xanthine oxidase during the midfollicular and midluteal phases of the menstrual cycle. These intervals were selected for examination on the basis of the significant differences that occur in estradiol and progesterone concentrations. Although it would have been of benefit to phenotype our subjects just before ovulation (when estradiol concentrations are highest), the free-living conditions of our subjects precluded our ability to accomplish this. Nonetheless, any effect of estradiol on CYP1A2, NAT2, or xanthine oxidase activity should have been evident within the first few days after ovulation.[40]

CYP1A2, NAT2, and xanthine oxidase activity and intraindividual variability were not statistically different in the midfollicular and midluteal phases of the menstrual cycle. Because serum hormone concentrations were not obtained for our subjects, the time frame for performance of phenotyping was restricted to minimize any hormonal concentration differences. Although we detected a significant sex difference among poor metabolizers of NAT2, statistical power with seven women and six men was only 48%,suggesting that this finding may not reflect a truly significant difference.

CYP1A2, NAT2, and xanthine oxidase activity remained relatively stable over 3 months. However, for CYP1A2, data for one subject appeared to be discrepant from those for the rest of the study population and were not adequately explained by study conditions or historical assessment of dietary and environmental factors. To verify these data, we examined caffeine urinary metabolic ratios after reanalysis of the urine specimens that confirmed the initial values. It is therefore possible that unknown exogenous or endogenous influences on CYP1A2 activity, not controlled for or anticipated, caused this apparently discordant pattern.

The quantitation of intraindividual variability in CYP1A2, NAT2, and xanthine oxidase activity is an important determination because it forms the basis for power and sample size calculations for future clinical investigations. With the standard deviations quantified for the repeated CYP1A2, NAT2, and xanthine oxidase activity measurements, we were able to calculate the sample size needed to detect menstrual cycle phase and sex differences. For the differences in CYP1A2, NAT2 extensive and poor metabolizers, and xanthine oxidase activities between the menstrual cycle phases to be statistically significant (with $\alpha = 0.05$ and $\beta = 0.2$), 77, 285, 52, and 200 women, respectively, would be needed. Power calculations revealed that our analyses had 80% power to detect a 25% difference in CYP1A2 metabolic ratios and a 10% difference in NAT2 and xanthine oxidase ratios. For the differences among CYP1A2, NAT2 extensive metabolizers, and xanthine oxidase indices between men and women to be significant, 2800, 50, and 394 subjects, respectively, would be needed. Power calculations revealed that our analyses had 80% power to detect a 35% difference in CYP1A2 metabolic ratios and 10% differ-

ence in xanthine oxidase ratios between men and women. The large numbers required to find menstrual cycle or sex differences would appear to support the lack of clinically significant menstrual cycle and sex differences in CYP1A2, NAT2, and xanthine oxidase activity for pharmacokinetic studies involving fewer than 50 subjects.

Within subjects, mean caffeine metabolic ratios for CYP1A2, NAT2 extensive and poor metabolizers, and xanthine oxidase activities varied by approximately 20%, 3%, 15%, and 5%, respectively. Because CYP1A2 and NAT2 poor metabolizer indices may fluctuate up to 50% and 30%, respectively, repeated baseline and treatment phenotyping assessments should be obtained for accurate determination of the effect of a given drug on activity. Our data showed that NAT2 extensive metabolizer indices and xanthine oxidase indices are extremely stable and most likely do not require repeat baseline and treatment measures. These baseline variability data should be taken into account when determining whether changes in metabolic ratio measures should be considered of clinical importance.

We concluded that menstrual cycle phase does not significantly affect CYP1A2, NAT2, or xanthine oxidase activity. Because differences in activity were not found between men and women, it appears that stratification need not be done on the basis of sex for pharmacokinetic investigations of drugs that are CYP1A2, NAT2, or xanthine oxidase substrates. However, it may be necessary, to stratify for large clinical or pharmacokinetic studies on the basis of the aforementioned power calculations. Use of the intraindividual variability data presented will provide more accurate estimates of sample size and power of clinical investigations involving CYP1A2, NAT2, and xanthine oxidase substrates.

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