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## **TITLE PAGE**

### **Drug Metabolizing Enzyme Inhibition by Ketoconazole Does Not Reduce Interindividual Variability of CYP3A Activity as Measured by Oral Midazolam**

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## **RUNNING TITLE PAGE**

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## ABSTRACT

Variable interindividual expression of cytochrome P450 (CYP) 3A presents a challenge in dosing drugs. Potent inhibitors of CYP3A such as ketoconazole have been explored to reduce the clearance of CYP3A substrates, thereby resulting in smaller dose requirements; however the impact of CYP3A inhibition on interindividual variability has not been well characterized. Our objective was to examine the effect of ketoconazole inhibition on CYP3A metabolic variability as measured by the CYP3A biomarker oral midazolam. A single dose of midazolam (0.075 mg/kg) was administered to 19 healthy Caucasian adults ( $38.7 \pm 8.8$  yrs, 9M/10F) at baseline and concurrently with ketoconazole (400 mg daily for 10 days) on day 6 or 9 of ketoconazole. Plasma samples were collected over 6-30 hours. A paired t-test and percent coefficient of variation (CV%) were used to evaluate differences in midazolam clearance and interindividual variability during both phases. Monte Carlo simulation was performed to determine probability distribution of area under the concentration-time curves (AUCs). Midazolam apparent oral clearance (CL/F) decreased by 89% ( $p < 0.0001$ ) during inhibition.  $C_{max}$  increased from 23 ng/mL (95%CI 19-29 ng/mL) to 55 ng/mL (95%CI 46-66 ng/mL),  $p < 0.0001$ . CV% increased from 41% to 58% from baseline to ketoconazole inhibition. AUC [median; range] were (0.20 mg•min/mL; 0.05 – 0.81 mg•min/mL) and (1.94 mg•min/mL; 0.25 – 25.4 mg•min/mL) at baseline and inhibition phase, with CV% of 41% and 61%, respectively. Ketoconazole decreased CYP3A activity but did not reduce interindividual variability.

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Use of a CYP3A inhibitor to standardize dosing of CYP3A substrates may not be feasible in clinical practice.

## **INTRODUCTION**

The cytochrome P450 (CYP) 3A subfamily comprises the most clinically important metabolic enzymes involved in drug metabolism. CYP3A exhibits broad substrate specificity, metabolizing nearly half of all marketed drugs (Gibbs and Hosea, 2003). This group is also the most abundant of the CYP isozymes, accounting for 29% and 70% of the CYPs in the liver and intestinal mucosal, respectively (Guengerich, 1991; Watkins, 1992). Currently, four isoforms have been described in humans: CYP3A4, CYP3A5, CYP3A7, and CYP3A43, of which the latter two are believed to have only a minor role in CYP3A mediated biotransformation (Daly, 2006).

The highly variable expression/function of CYP3A observed between individuals poses a therapeutic challenge. Intersubject variability in CYP3A mediated activity has been reported to be large (approximately 10-fold difference). Some studies have reported variability of greater than 20-fold (Lin et al., 2001; Zhu et al., 2003). An obvious challenge is in optimizing drug therapy for maximization of efficacy while limiting toxicity. This is particularly a concern for drugs with narrow therapeutic indices, including many immunosuppressants and chemotherapeutic agents. Some studies have explored the use of potent CYP3A inhibitors such as the imidazole antifungal agent, ketoconazole, to increase exposure of drugs such as in cyclosporine at reduced doses. Usually the intention of CYP inhibition in these studies has been to allow use of smaller

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doses and thereby decrease cost (Martin et al., 1999). However, investigation of the effect of inhibition on interindividual variability has not been well established.

Midazolam, a short acting benzodiazepine, is selectively metabolized by CYP3A, specifically CYP3A4 and CYP3A5, to the major metabolite 1'hydroxymidazolam (Tsunoda et al., 1999). It is widely used as a phenotyping probe for CYP3A activity. Apparent oral clearance of midazolam is the pharmacokinetic parameter that is recognized as the biomarker for hepatic and intestinal CYP3A activity.

The purpose of this study is to examine whether the large intersubject variability in observed CYP3A activity can be significantly decreased with use of the potent CYP3A inhibitor ketoconazole.

## **METHODS**

The data for this analysis were obtained from a previously published study (Chung et al., 2006). Only the data from the midazolam phenotyping phases of baseline and inhibition were used. The study was approved by the Institutional Review Board of Bassett Healthcare. All subjects provided written informed consent prior to study procedures.

### **Subjects**

Twenty healthy Caucasian adults between the ages of 18 to 55 years participated in the study. Subjects were required to have a body mass index (BMI) between 18 to 30 kg/m<sup>2</sup>. They avoided tobacco or nicotine containing products for at least 3 months and were

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determined to be in good health by complete medical history, physical examination, electrocardiogram, and clinical laboratory tests. Women were required to be non-pregnant, non-nursing, pre-menopausal and either surgically sterile or using acceptable non-hormonal methods of contraception at least 14 days prior to and until 30 days after completion of the study. Urine pregnancy tests were performed for all women of childbearing potential during the screening phase and prior to each dosing period. Use of any medication, herbal preparation or nutritional supplement known to affect CYP3A activity within 7 days or 5 half-lives (whichever was longer) prior to first dose of study medication was prohibited. Subjects were required to refrain from grapefruit, apple, and orange juice containing products for 7 days prior to study initiation and for the duration of the study. Subjects with a history of regular alcohol consumption exceeding 1 drink per day within 6 months of screening or intolerance/hypersensitivity to benzodiazepines or imidazoles were excluded.

### **Study Design and Procedures**

The study was a sequential, open-label crossover trial using oral midazolam as a biomarker for CYP3A activity at baseline and after inhibition. The full protocol has been previously described (Chung et al., 2006), and is briefly described below.

Each subject completed the baseline phase then proceeded to the inhibition phase following a washout period of at least 1 week. Subjects arrived at the research unit between 0600 and 1000 hours following an overnight fast of at least 8 hours. An intravenous cannula was placed in the forearm for serial blood draws. During the baseline

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phase, a single oral dose of midazolam 0.075 mg/kg (Versed<sup>®</sup> 2mg/mL syrup, Roxane Laboratories, Inc., Columbus, OH) was administered alone. Blood samples (7 mL) were collected at predose, 0.5, 1, 1.5, 2, 3, 4, and 6 hours after midazolam administration.

In the inhibition phase, oral ketoconazole 400 mg (two ketoconazole 200 mg tablets, Taro Pharmaceuticals, Hawthorne, NY) was self-administered daily for 10 days. Ketoconazole tablets were dispensed in unit dose packages. Subjects were instructed to take the dose in the morning at the same time daily. Adherence to the ketoconazole regiment was checked by unit dose package count. A single oral dose of midazolam 0.075 mg/kg was administered on either day 6 or day 9 of ketoconazole 2 hours before ketoconazole administration. Randomization of midazolam to day 6 or day 9 was done because of additional probe administration in the original study. However, since peak inhibition with ketoconazole occurs after 48 hours of continuous dosing, subjects were assumed to be inhibited at either study day. Blood samples for midazolam concentrations were collected at predose, 0.5, 1, 2, 4, 6, 8, 12, 24 and 30 hours after midazolam dosing.

Subjects were required to remain seated for a minimum of 2 hours post midazolam administration. All blood samples were immediately centrifuged at approximately 2800 rpm at 4°C for 15 minutes. Harvested plasma samples were kept frozen at -80°C until analysis. Vital signs and oxygen saturation by pulse oximetry were monitored for 2 hours after midazolam administration during each phase.

### **Assay and Pharmacokinetic Analysis**

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Midazolam plasma concentrations were analyzed at Prevalere™ Life Sciences, Inc., Whitesboro, NY. Liquid chromatography tandem mass spectrometry (LC-MS-MS) using alprazolam as the internal standard was used. The assay has been previously described (Kashuba et al., 1998; Chung et al., 2006). Pharmacokinetic parameters were analyzed by noncompartmental analysis using WinNonlin® version 3.2 (Pharsight Corporation, Mountain View, CA) (Chung et al., 2006). Apparent oral total body clearance (CL/F where F= bioavailability) data are presented as weight adjusted clearance. A 10,000-subject Monte Carlo simulation using the mean and standard deviation of weight adjusted CL/F and a log-normal distribution was performed to determine probability distribution of area under the concentration-time curves (AUCs) using Crystal Ball® version 7 [Decisioneering, Inc., Denver, CO]. AUC was simulated using the equation  $AUC = F \cdot \text{dose} / CL$ . A midazolam dose of 5mg was chosen for the oral midazolam simulation study. The Monte Carlo simulation distributions were reviewed and confirmed to be an accurate reflection of the data used to generate them.

### **Statistical Analysis**

*A priori* sample size calculations determined that 16 subjects were required to detect a minimum difference of 25% in area under the concentration-time curve  $AUC_{(0-\infty)}$  between phases with 80% power and an alpha of 0.05. SYSTAT® version 11 (Systat Software Inc., Point Richmond, CA) was used for statistical analyses. Log transformed CL/F, peak concentrations (Cmax) and  $AUC_{(0-\infty)}$  between phases was evaluated using a paired t-test. An F test was used to compare variance in midazolam apparent oral clearance during baseline and inhibition phases. Percent coefficient of variation (CV%)



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was calculated to assess interindividual variability in CL/F in each phase. Data are presented as geometric mean with 95% confidence interval (CI). Data for the Monte Carlo simulation is presented as the median and the range. P value of <0.05 was considered significant.

## RESULTS

Twenty subjects (all Caucasians) were enrolled in the study. Nineteen subjects (10 women, 9 men) completed both baseline and inhibition phases. One male subject withdrew during the inhibition phase because of gastrointestinal adverse effects attributed to ketoconazole. Demographic data for the 19 subjects are shown in Table 1. Both phases were well tolerated. Sixteen of 19 subjects were homozygous for CYP3A5\*3/\*3 and the other 3 were heterozygous. No clinically significant adverse events were noted.

Mean midazolam plasma concentration versus time at baseline and inhibition are shown in Figure 1. Co-administration of ketoconazole, a potent inhibitor of CYP3A, increased midazolam C<sub>max</sub> from a geometric mean 23 ng/mL (95% CI 19-29 ng/mL) to 55 ng/mL (95% CI 46-66 ng/mL),  $p < 0.0001$ . CV% increased from 41% to 58% from baseline to ketoconazole inhibition. Midazolam AUC<sub>(0-∞)</sub> increased 9.5 fold from 49 ng·hr/mL (95% CI 40-60 ng·hr/mL) to 467 ng·hr/mL (95% CI 370-589 ng·hr/mL),  $p < 0.0001$  with CV% of 41% and 61%, respectively. Midazolam apparent oral clearance decreased by a mean of 89% ( $p < 0.0001$ ) as shown in Table 2. There were no differences in midazolam CL/F on the basis of the order of drug administration during any phases. Figure 2 displays the drop in midazolam CL/F from baseline to inhibition phase. Variance in CL/F also

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decreased from 128.8 to 3.1 from baseline to inhibition phase, representing a decrease in variance of approximately 98% ( $p < 0.001$ ).

Monte Carlo simulation distributions of area under the concentration-time profiles of midazolam at baseline and ketoconazole inhibition are displayed in Figures 3A and 3B, respectively. Estimated AUC [median; range] were (0.20 mg•min/mL; 0.05 – 0.81 mg•min/mL) and (1.94 mg•min/mL; 0.25 – 25.4 mg•min/mL) at baseline and inhibition phase, respectively. CV% was 61% at inhibition versus 41% at baseline.

## DISCUSSION

Ketoconazole is a known potent inhibitor of CYP3A activity. We investigated whether the use of ketoconazole (at the recommended inhibitory dose of 400 mg daily) (Bjornsson et al., 2003; Chien et al., 2006) would result in significant reduction in intersubject variability of CYP3A isozyme activity. Consistent with other findings,  $C_{max}$  and  $AUC_{(0-\infty)}$  increased 2.4 and 9.5 fold, respectively, in our study (Olkola et al., 1996). Apparent oral clearance decreased approximately 9-fold for all subjects and did not differ by sex. Those subjects with the highest clearance at baseline exhibited the greatest absolute change in clearance. The relative (percent) decrease in clearance was similar for each subject. The range in the decrease of  $CL/F$  was 85% to 94%. Subjects with the highest CYP3A activity at baseline continued to have the highest CYP3A activity during inhibition (Figure 2). This illustrates that inhibition by ketoconazole, while reducing CYP3A activity in all subjects, did not convert all subjects to an equivalent level of CYP3A activity. Although variance of  $(CL/F)$  decreased as expected in the inhibition

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phase, the measure of variability between individuals (CV%) actually increased. Interestingly, the increase in variability was seen only in women (Table 2). CV% for men remained constant at 28% from baseline to ketoconazole inhibition, whereas CV% increased from 28% to 51% for females. Whether this observation is unique to our subjects or a sex-difference in CYP3A activity is unknown, however, our previous data has suggested that women show modestly greater CYP3A isozyme activity compared to men when using oral midazolam as a probe (Chen et al., 2006).

Monte Carlo simulations were performed to determine the distribution of exposure using a 10,000 subject simulation. As shown in Figures 3A and 3B, the range in exposure to midazolam increased from baseline to inhibition, consistent with findings from midazolam CL/F data showing an increase in interindividual variability with CYP3A inhibition.

CYP3A inhibitors such as ketoconazole have been used in an attempt to “standardize dose” of certain CYP3A substrates. Martin and colleagues (Martin et al., 1999) evaluated the use of ketoconazole as an inhibitor of cyclosporine to reduce the dose and thus the cost of therapy. Tham et al. investigated the use of ketoconazole to reduce variability in clearance of midazolam and docetaxel (Tham et al., 2006). These authors reported a reduction in the variability in midazolam clearance but not of docetaxel in two separate groups of patients assessed during a constitutive state or during inhibited activity. A recent study using high dose ketoconazole (400 mg three times a day) found intersubject variability of docetaxel (a drug that is not solely a CYP3A substrate) increased with

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inhibition (Engels et al., 2006). Another study by Desai and colleagues observed no significant decrease in interpatient variability of carboxyamidatriazole (CYP3A substrate) when a 200 mg dose of ketoconazole was co-administered (Desai et al., 2004).

The cause for the wide intersubject variability observed with CYP3A activity has not been fully elucidated. Factors such as age, sex, and genetic polymorphism of CYP3A5 have been investigated in an attempt to explain differences in CYP3A activity. Although some findings suggest these factors may play a role, knowledge in this area remains limited. Recent data from Bosch and colleagues (Bosch et al., 2006) showed that several single nucleotide polymorphisms (SNPs) are found in the pregnane X receptor (PXR) gene. The PXR receptor has been implicated in the up-regulation of CYP3A. These authors suggest that these SNPs may influence the PXR receptor and potentially CYP3A activity.

The concept of using an inhibitor to reduce the intersubject variability of CYP3A activity is an interesting one. Potentially, if inhibition resulted in individuals having a relatively “similar” clearance of a CYP3A substrate, standardized dosing could be used to achieve a narrow range of exposure. Our data suggest that inhibition of CYP3A activity with ketoconazole does not reduce intersubject variability. In fact, between subject variability actually increased with CYP3A inhibition. Use of a potent inhibitor in a clinical setting does not reduce the variability of CYP3A substrate exposure and thus, does not allow for use of a “one dose fits all” approach.

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## REFERENCES

- Bjornsson TD, Callaghan JT, Einolf HJ, Fischer V, Gan L, Grimm S, Kao J, King SP, Miwa G, Ni L, Kumar G, McLeod J, Obach RS, Roberts S, Roe A, Shah A, Snikeris F, Sullivan JT, Tweedie D, Vega JM, Walsh J and Wrighton SA (2003) The conduct of in vitro and in vivo drug-drug interaction studies: a Pharmaceutical Research and Manufacturers of America (PhRMA) perspective. *Drug Metab Dispos* **31**:815-832.
- Bosch TM, Deenen M, Prunzel R, Smits PH, Schellens JH, Beijnen JH and Meijerman I (2006) Screening for polymorphisms in the PXR gene in a Dutch population. *Eur J Clin Pharmacol* **62**:395-399.
- Chen M, Ma L, Drusano GL, Bertino JS, Jr. and Nafziger AN (2006) Sex Differences in CYP3A Activity Using Intravenous and Oral Midazolam, in: *American Society for Clinical Pharmacology and Therapeutics*, pp 66, Clinical Pharmacology and Therapeutics, Baltimore, MD.
- Chien JY, Lucksiri A, Ernest CS, 2nd, Gorski JC, Wrighton SA and Hall SD (2006) Stochastic prediction of cyp3a-mediated inhibition of midazolam clearance by ketoconazole. *Drug Metab Dispos* **34**:1208-1219.
- Chung E, Nafziger AN, Kazierad DJ and Bertino JS, Jr. (2006) Comparison of midazolam and simvastatin as cytochrome P450 3A probes. *Clin Pharmacol Ther* **79**:350-361.
- Daly AK (2006) Significance of the minor cytochrome P450 3A isoforms. *Clin Pharmacokinet* **45**:13-31.

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- Desai AA, Innocenti F, Janisch L, DeMario M, Shepard D, Ramirez J, Fleming GF and Ratain MJ (2004) A phase I trial of pharmacokinetic modulation of carboxamidotriazole (CAI) with ketoconazole in patients with advanced cancer. *Cancer Chemother Pharmacol* **54**:377-384.
- Engels FK, Mathot RA, Loos WJ, van Schaik RH and Verweij J (2006) Influence of High-Dose Ketoconazole on the Pharmacokinetics of Docetaxel. *Cancer Biol Ther* **5**.
- Gibbs MA and Hosea NA (2003) Factors affecting the clinical development of cytochrome p450 3A substrates. *Clin Pharmacokinet* **42**:969-984.
- Guengerich FP (1991) Reactions and significance of cytochrome P-450 enzymes. *J Biol Chem* **266**:10019-10022.
- Kashuba AD, Bertino JS, Jr., Rocci ML, Jr., Kulawy RW, Beck DJ and Nafziger AN (1998) Quantification of 3-month intraindividual variability and the influence of sex and menstrual cycle phase on CYP3A activity as measured by phenotyping with intravenous midazolam. *Clin Pharmacol Ther* **64**:269-277.
- Lin YS, Lockwood GF, Graham MA, Brian WR, Loi CM, Dobrinska MR, Shen DD, Watkins PB, Wilkinson GR, Kharasch ED and Thummel KE (2001) In-vivo phenotyping for CYP3A by a single-point determination of midazolam plasma concentration. *Pharmacogenetics* **11**:781-791.
- Martin JE, Daoud AJ, Schroeder TJ and First MR (1999) The clinical and economic potential of cyclosporin drug interactions. *Pharmacoeconomics* **15**:317-337.

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Olkola KT, Ahonen J and Neuvonen PJ (1996) The effects of the systemic antimycotics, itraconazole and fluconazole, on the pharmacokinetics and pharmacodynamics of intravenous and oral midazolam. *Anesth Analg* **82**:511-516.

Tham LS, Goh BC, Wang LZ, Yong WP, Wong CI, Lee SC, Soo R, Sukri N and Lee HS (2006) Ketoconazole inhibition of CYP3A activity made midazolam but not docetaxel pharmacokinetics more predictable, in: *American Society for Clinical Pharmacology and Therapeutics* (Therapeutics CPa ed, pp 23, Baltimore, MD.

Tsunoda SM, Velez RL, von Moltke LL and Greenblatt DJ (1999) Differentiation of intestinal and hepatic cytochrome P450 3A activity with use of midazolam as an in vivo probe: effect of ketoconazole. *Clin Pharmacol Ther* **66**:461-471.

Watkins PB (1992) Drug metabolism by cytochromes P450 in the liver and small bowel. *Gastroenterol Clin North Am* **21**:511-526.

Zhu B, Liu ZQ, Chen GL, Chen XP, Ou-Yang DS, Wang LS, Huang SL, Tan ZR and Zhou HH (2003) The distribution and gender difference of CYP3A activity in Chinese subjects. *Br J Clin Pharmacol* **55**:264-269.



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## FOOTNOTES

- a) This manuscript was presented in part at the 107<sup>th</sup> Annual Meeting of the American Society for Clinical Pharmacology and Therapeutics in Baltimore, MD (March 11, 2006) and at the Food and Drug Administration Science Forum in Washington, DC (April 18, 2006)
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## FIGURE LEGENDS

**Figure 1.** Midazolam plasma concentration (mean  $\pm$  SD) versus time profile at baseline (  $\cdots\blacktriangle\cdots$  ) and ketoconazole inhibition (  $\text{---}\blacksquare\text{---}$  ).

**Figure 2.** Midazolam apparent oral clearance at baseline and after ketoconazole inhibition in women (  $\cdots\blacktriangle$  ) [N=10] and men (  $\text{---}\blacksquare$  ) [N=9]. Mean clearance for women and men at each phase is shown as a *solid bar*  $\pm$  SD. **W** denotes women and **M** denotes men.

### Figure 3.

**Panel A.** Distribution of area under the concentration-time curves of oral midazolam administration at baseline via 10,000 subject Monte Carlo simulation.

**Panel B.** Distribution of area under the concentration-time curves of oral midazolam after ketoconazole inhibition via 10,000 subject Monte Carlo simulation.

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**TABLE 1. Demographics, Laboratory and Weight-Based Oral Midazolam Dose for 19 Healthy Volunteers**

	<b>Total</b>	<b>Women</b>	<b>Men</b>
Subjects (N)	19	10	9
Age (yr)	38.7 ± 8.8	38.7 ± 5.8	38.7 ± 11.6
Weight (kg)	73.4 ± 11.3	66.3 ± 8.9	81.3 ± 8.1
Body mass index (kg/m <sup>2</sup> )	25.5 ± 2.8	24.6 ± 3.2	26.4 ± 2.1
Midazolam dose (mg)	5.5 ± 0.8	5.0 ± 0.7	6.1 ± 0.6
SCr (mg/dL)	0.9 ± 0.1	0.8 ± 0.1	1.0 ± 0.1
AST (U/L)	24.9 ± 8.2	20.9 ± 5.2	29.4 ± 8.8
ALT (U/L)	29.4 ± 7.4	25.5 ± 6.8	33.8 ± 5.4

Data presented as arithmetic mean ± SD.

SCr: Serum Creatinine

AST: Aspartate aminotransferase

ALT: Alanine aminotransferase

**TABLE 2. Apparent Oral Midazolam Clearance by Sex as a Measure of CYP3A Activity at Baseline and after Ketoconazole****Inhibition**

	<b>Total (N=19)</b>	<b>Women (N=10)</b>	<b>Men (N=9)</b>
<b>Midazolam CL/F (mL/min/kg)</b>			
<i><u>Baseline Phase</u></i>			
Mean (95% CI)	25.4 (20.7 – 31.0)	33.9 (27.9 – 41.2)	18.3* (14.7 – 22.9)
Range	12.2 – 56.6	21.1 – 56.6	12.2 – 28.8
Variance	128.8	95.8	28.9
CV%	41	28	28
<i><u>Inhibition Phase</u></i>			
Mean (95% CI)	2.7** (2.1 – 3.4)	3.6 (2.6 – 4.9)	1.9* (1.6 – 2.4)
Range	1.2 – 8.5	2.2 – 8.5	1.2 – 3.2
Variance	3.1**	4.0**	0.3**
CV%	58	51	28

Data presented as geometric mean

$$\text{CV\%} = \frac{\text{SD}}{\text{arithmetic mean}} \times 100\%$$

\* p<0.01 versus women

\*\* p<0.0001 versus baseline

Figure 1

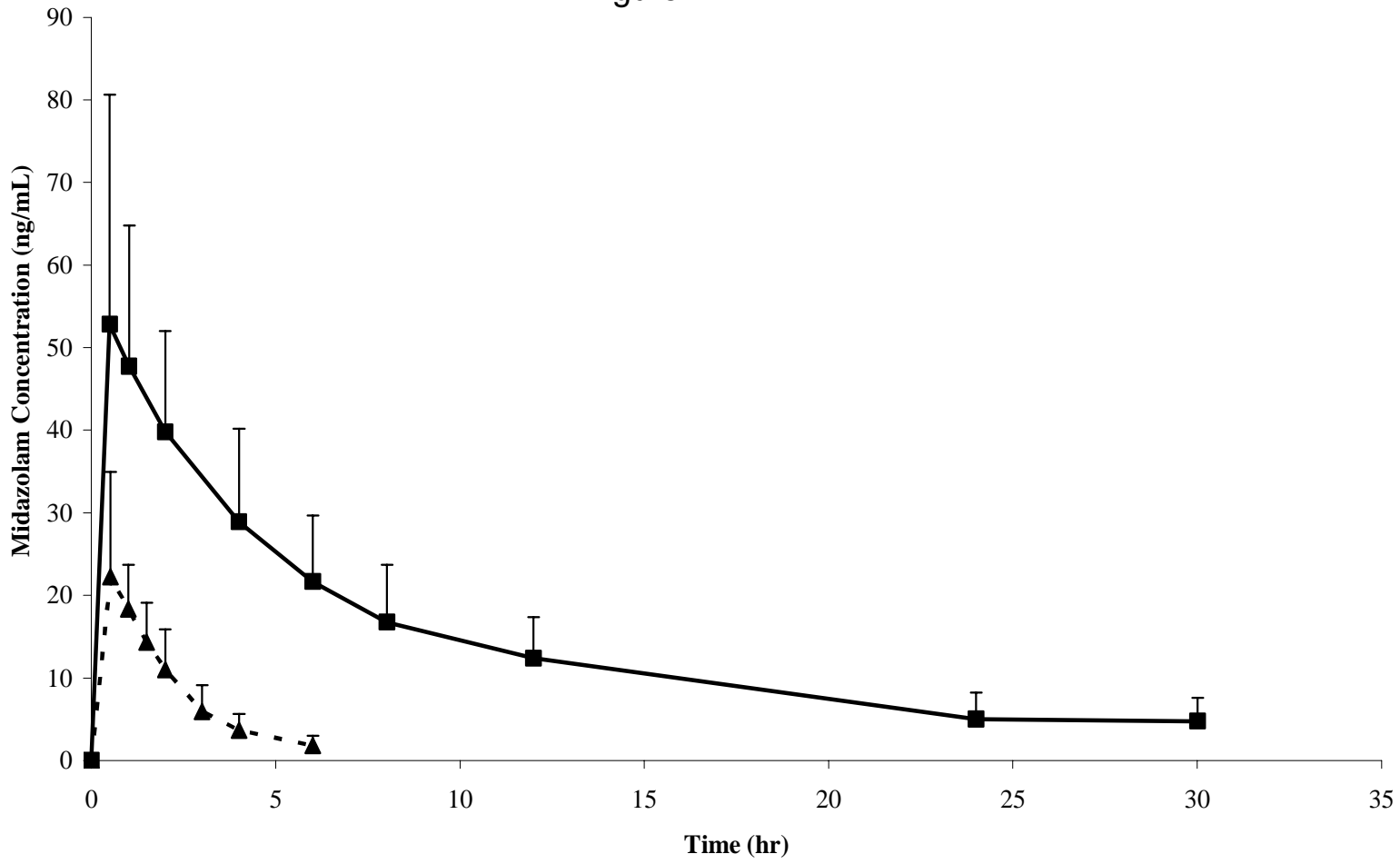


Figure 2

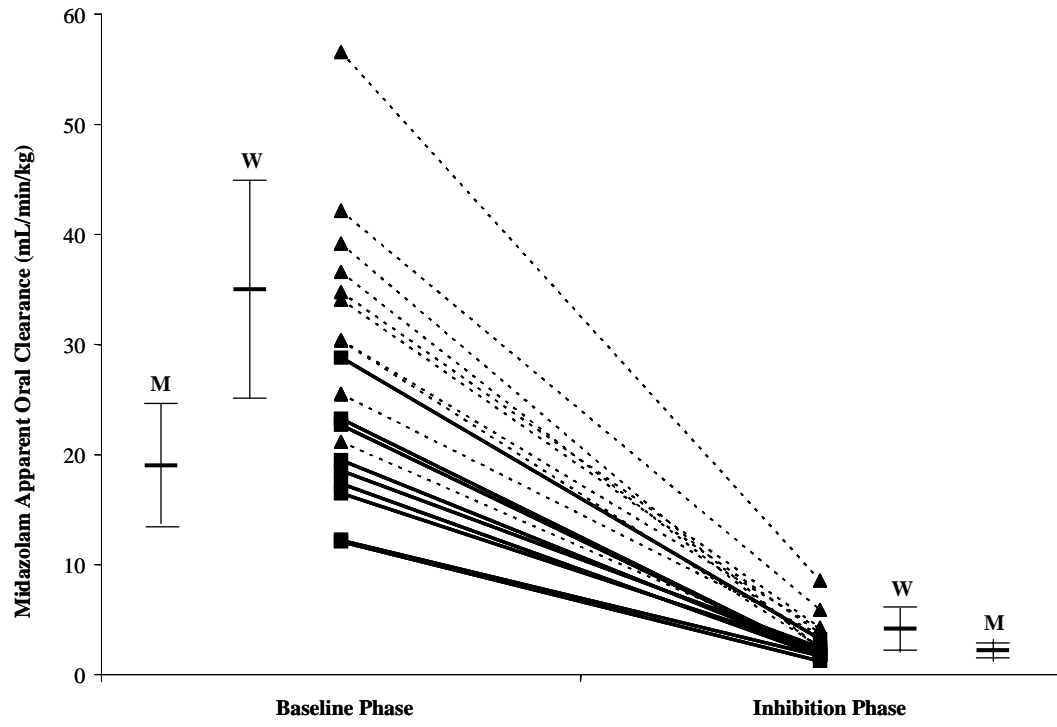
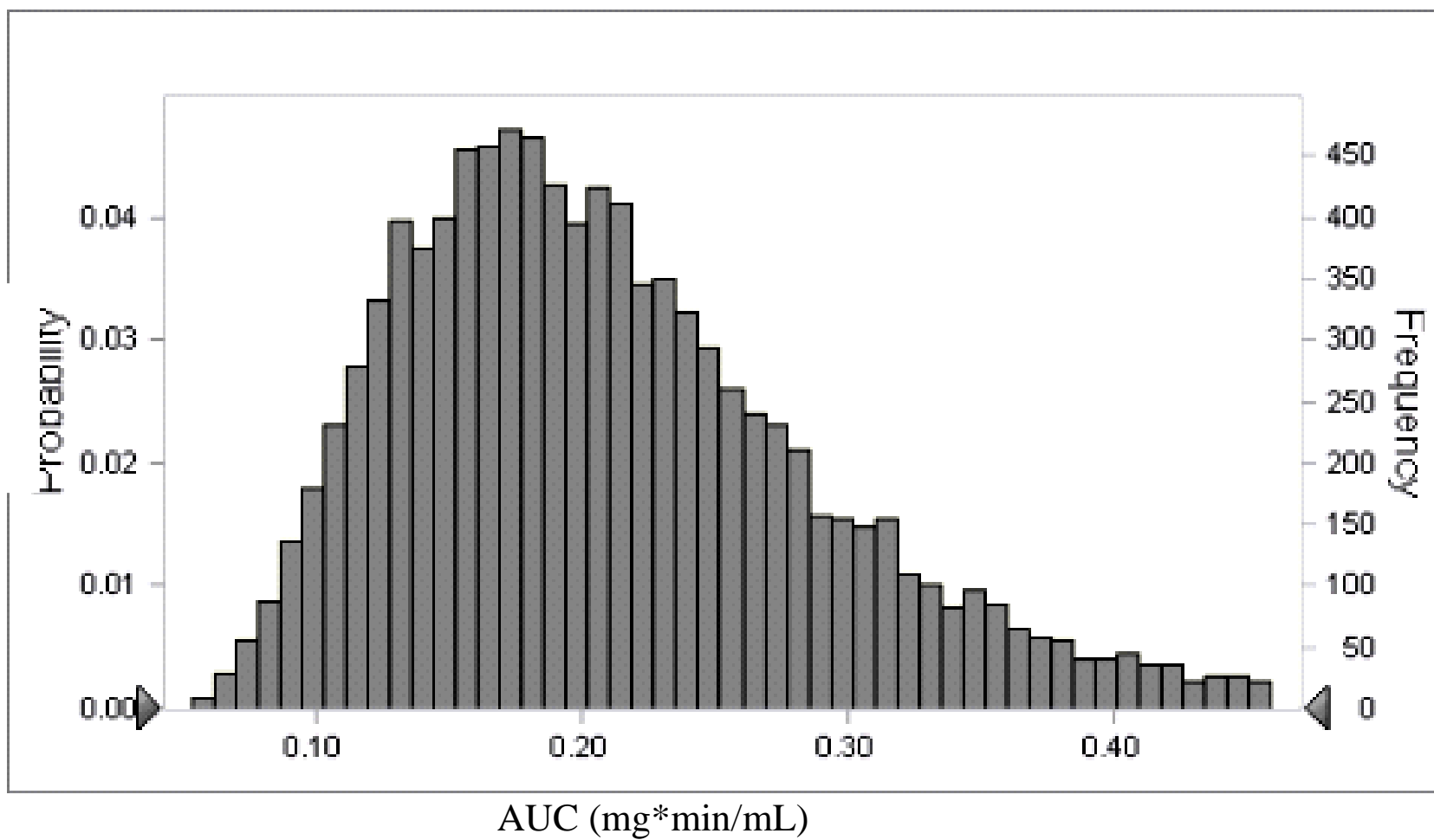


Figure 3A.





**Figure 3B.**

