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# Mild Hypothermia Alters Midazolam Pharmacokinetics in Normal Healthy Volunteers

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# Running Title: Midazolam pharmacokinetics during mild hypothermia in human

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*Non-standard abbreviations:* Cytochrome P450 (CYP450), Electrocardiography (ECG), Liquid chromatography-single quadrupole mass spectrometry (LC/MS), Pharmacokinetics (PK), Systemic clearance (CLs), Area under curve (AUC)

**ABSTRACT**

The clinical use of induced hypothermia has been rapidly expanded due to evidences of hypothermia neuroprotective effect. Despite its clinical usage, the effect of hypothermia on specific pathways of drug elimination in human is relatively unknown. In order to gain insight into the potential effects of hypothermia on drug metabolism and disposition, we evaluated the pharmacokinetics of midazolam as a probe for cytochome CYP3A4/5 activity during mild hypothermia in human volunteers. A second objective of this work was to determine whether benzodiazepine and magnesium administrated intravenously would facilitate the hypothermia induction. Subjects were enrolled in a randomized cross-over study design which included two mild hypothermia groups (4 °C saline infusions and 4 °C saline+magnesium) and two normothermia groups (37 °C saline infusions and 37 °C saline+magnesium). The lowest temperature achieved in the 4 °C saline+magnesium and 4 °C saline infusions were 35.4 °C ± 0.4 °C and 35.8 °C ± 0.3 °C, respectively. A significant decrease in the formation clearance of the major metabolite 1'-hydroxymidazolam was observed during the 4 °C saline+magnesium compared to the 37 °C saline group ($p<0.05$). Population pharmacokinetic modelling identified a significant relationship between temperature and clearance (CL) and inter-compartmental clearance (Q) for midazolam. This model predicted midazolam clearance decreases 11.1% for each degree Celsius reduction in core temperature from 36.5 °C. Midazolam with magnesium facilitated the induction of hypothermia but shivering was minimally suppressed. These data provided proof of concept that even mild and short duration changes in body temperature significantly affect midazolam metabolism. Future studies in patients who receive lower and longer duration of hypothermia are warranted.

## Introduction

Mild hypothermia has been employed clinically as a neuroprotective strategy in a wide array of diseases including cardiac arrest, stroke, hypoxic-ischemic encephalopathy, hepatic encephalopathy and traumatic brain injury (Bernard et al., 2002; Hypothermia after Cardiac Arrest Study Group, 2002; Marion et al., 2002; Kim et al., 2009). Patients are typically cooled to 32-34°C for a duration of 12-48 hours in adults and up to 72 hours in neonates. This degree of cooling has been shown to alter drug concentration and response of several medications, such as phenobarbital, vecuronium, phenotoyin, propofol, fentanyl and morphine etc. (Kadar et al., 1982; Caldwell et al., 2000; Iida et al., 2001; Heier et al., 2002; Leslie et al., 1995; Fritz et al., 2005) The mechanism of alterations in drug level and response with temperature has not been fully elucidated. Given the high rate of adverse drug events and the difficulty indentifying such events in critical ill patients, determining the key factors that alter drug disposition is essential for safe and effective pharmacotherapy.

Previous experiments in animal models as well as in human studies by our laboratory and others have implicated reduced hepatic metabolism as a mechanism of increased drug levels during cooling. Medications commonly used in critical ill patients (antiarrythmics, β-blockers, calcium channel blockers, benzodiazepines, anesthetics, opioids, anticonvulsnats, and proton pump inhibitors, etc) are largely metabolized through CYP450 system and specifically by CYP3A4/5 isoform (Tortorici et al., 2007). Studies to identify the effects of mild hypothermia on CYP450 metabolism in humans are limited and the translational significance of the observations in the rat model remains to be identified. The primary objective of our study was to evaluate the effect of temperature reduction on CYP3A4/5 activity in healthy human subjects by determining the alterations in the metabolism of midazolam, a well known CYP3A4/5 probe in human.

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A secondary objective of our study was to determine if benzodiazepine administration with magnesium would facilitate the induction of therapeutic hypothermia in conscious patients. Hypothermia is currently achieved through methods that force core temperature below the internal homeostatic set point. Forced cooling induces compensatory responses such as shivering and vasoconstriction which are an obstacle to reaching target clinical hypothermic temperatures prior to hospital admission (Nagao et al., 2000; Hayashi et al., 2000; Kim et al., 2009; Polderman and Herold, 2009). Magnesium sulfate (MgSO4) has been shown to facilitate cooling and blunt the shivering response associated with cold saline infusion (Zweifler et al., 2004; Wadhwa et al., 2005). In the current study, we chose a factorial design to examine the effects of administration of cold saline in conjunction with benzodiazepine sedation and magnesium sulfate induced vasodilation as a method to induce hypothermia in concise subjects.

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## Methods

**Subjects.** This study was approved by the University of Pittsburgh Institutional Review Board. Six healthy male subjects between the ages 19-39 years provided informed consent and completed all phases of the study. Each subject received a standard history, physical examination, laboratory studies (serum electrolytes, renal and liver function, thyroid stimulating hormone, hemoglobin, and hematocrit) and a twelve-lead ECG to screen for the presence of cardiac or any other underlying disease. Subjects were excluded if they had an abnormal laboratory value, any known medical problems, or if they were taking any medications with the exception of seasonal allergy medication, over the counter non-steroidal anti-inflammatory drugs, or acetaminophen. Other exclusion criteria included a history of cardiac disease in a family member under the age of 40, allergy to midazolam or other benzodiazepine/narcotic medication, current smoker or recreational drug user, and a body mass index > 35. Body fat percentage was measured by three site skinfold analysis prior to the first protocol visit (Pollack et al., 1980). The subjects were asked not to consume grapefruit juice and herbal dietary supplements including St. John's wort from two days before each study day. Alcoholic drinks and caffeine-containing food and beverages were also not allowed 24 hours before or during the study.

**Testing Protocol.** We conducted a prospective, randomized, single center, four-way crossover laboratory study. Testing was performed in the Emergency Responder Human Performance Lab under the supervision of a study physician. Mild hypothermia was induced by rapid cold saline infusion (a total infusion of 30mL/kg of saline over 30-minutes) with or without 4g magnesium sulfate (Moore et al., 2008). Normothermia group control was given the warm saline infusion at same speed with or without magnesium. Therefore, the four treatment groups were 1) 37ºC saline infusion (Warm), 2) 37ºC saline infusion with magnesium sulfate infusion (Warm+Mg), 3)

4ºC saline infusion (Cold), and 4) 4ºC saline infusion with magnesium sulfate infusion (Cold+Mg). When provided, magnesium was administered with the cold or warm saline infusion. The four visits were separated by at least one week. Subjects received standard three-lead electrocardiogram, blood pressure monitoring, pulse oximetry, and end-tidal carbon dioxide monitoring throughout each study visit. Blood pressure and subject thermal sensation were measured at five-minute intervals throughout the infusion and every ten minutes thereafter. Our study involved a minimal sedation from given midazoalm, a universal Ramsay sedation scale has been used to evaluate the sedation state (Remsay et al., 1974). An 18-gauge peripheral intravenous catheter was placed in the antecubital vein of each subject for the saline infusion. A second 18-gauge peripheral intravenous catheter was placed in a superficial arm or hand vein for blood sampling. Following the 30-minute saline infusion, a lorazepam infusion 1mg/hr was initiated attempting to maintain the lower core temperature for three hours until 210 minutes. Following the three-hour cooling maintenance period, the subject was covered with blankets and observed for an additional hour before being discharged from the lab. The room temperature in the laboratory was maintained at approximately 22°C throughout the study.

**Temperature Measurements.** Core body temperature was monitored using a pre-calibrated ingestible thermometer pill that continuously measures temperatures in the range of 0 to 50°C (HQ Technologies, Palmetto, Florida). Temperature values recorded by this device are intermediate to esophageal and rectal temperature (O'Brien et al., 1998). The capsule was administered to the subject 60 minutes prior to the beginning of the protocol with approximately 30 mL of water. The protocol was initiated after three consecutive measurements indicated a stable core temperature reading. Skin-surface thermistors were placed over 1) the clavicular head of pectoralis major, 2) supraspinatus, 3) triceps brachii, and 4) quadriceps femoris muscles. Core and skin surface temperatures were documented every two minutes during the infusion and

every ten minutes thereafter. Mean skin temperature (Tsk) was calculated by using the formula Tsk = chest (0.25) + back (0.25) + thigh (0.3) + arm (0.2) (Ayling, 1986). To mathematically describe the temperature reduction on these subjects, we estimated the area of temperature change as a reflection of cooling burden. Every cooling burden was calculated from individual time-temperature curves. (Trapezoid area from 0-210 minutes minus the area under temperature curve calculated from Prism).

**Drug Administration and Sampling.** Total 6mg midazolam was given intravenously by separating three times (2mg per dose). The three doses were given at zero, ten, and twenty minutes from starting saline infusion. Blood samples were collected at baseline and at 5, 15, 25, 30, 50, 80, 140, and 200 minutes. The 5, 15, 25, 30, 50, 80, 140 and 200 minutes sample were referring to the time after the first midazolam IV administration. Specifically, the 5 minutes sample and 15 minutes samples were prior to the $2^{nd}$ and $3^{rd}$ midazolam doses, respectively. The blood samples were centrifuged at 2000×g for 10 minutes. Total urine volume was measured at the end of rewarming and a urine aliquot was collected from the final volume for calculating formation clearance of the major metabolite 1'-hydroxymidazolam. All plasma and urine samples were frozen at −70°C until the time of analysis.

**Drug Assay.** Plasma concentrations of midazolam and urine concentration of 1'-hydroxymidazolam were determined by using liquid chromatography-single quadrupole mass spectrometry (LC/MS, ThermoFinnigan single quad MS). A total of 150μL of plasma was added to 0.5M $Na_2CO_3$/$NaHCO_3$ (pH=9.6) buffer solution. Samples were then spiked with 20μL of midazolam- duterated d4 (0.1μg/mL) (Cerilliant Corp, TX) as internal standard. After vortexing, 5mL methyl-terbutyl-ether was added to the sample, centrifuged at 3000×g, 4 °C for 10 minutes. The extracted supernatant were collected in a glass tube and evaporated to dryness under gentle nitrogen, followed by reconstitution in 200 μL of mobile phase which consisted of 0.1% formic

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acid in water and acetonitrile. A volume of 20μL of each sample was injected onto a Thermo RP18 5μm, 2.1 ×150mm HPLC column. The mass spectrometer was operated in the positive ion mode for detection of the protonated-molecular ions $[M + l]^+$ with a cone temperature of 350°C and a capillary voltage of 2200V. Positive ions at m/z for midazolam, 1'-hydroxymidazolam and midazolam-d4 were 325.9, 341.9 and 330.7, respectively. To measure 1'-hydroxymidazolam concentration in the urine, urine samples were incubated with 2000UI glucuronidase (Sigma, MI) with 0.2M acetate buffer (pH 4.9) at 37°C for 24 hrs. The extraction and LC/MS analytical procedure was similar to the midazolam method described above. Calibration curves ($r^2 \geq 0.99$) for midazolam (3-300ng/mL) and 1'-hydroxymidazolam (100-2000ng/mL) were linear. Midazolam and 1'-hydroxymidazolam analytical LC/MS methods have been validated with < 9.5% and <8.3% inter-day precision, and < 7.2% and < 7.3% intra-day precision over all concentrations, respectively. Recoveries of the midazolam and 1'-hydroxymidazolam from liquid-liquid extraction were > 89.2% and > 91.7% over all concentrations, respectively.

**Physiological Data Analyses.** Repeated temperature measurements (core and skin temperatures), heart rate, respiratory rate and end-tidal $CO_2$ were compared by generalized estimating equations using the factors of time, cold saline infusion, magnesium infusion, and the interaction of cold and magnesium. Estimated effect for each factor is presented as the coefficient estimate (B) with 95% confidence interval (95% CI). Analyses were performed with Prism release 4.0c.

**Non-compartmental Pharmacokinetic Analysis.** Non-compartmental analysis (WinNonlin professional Version 4.01, Pharsight, Mountain View, CA) was used to fit the time-concentration data and obtain the estimation for the area under the curve from time zero to infinity ($AUC_{0\text{-}inf}$), area under the curve from time zero to the last sample time ($AUC_{0\text{-}T}$), systemic clearance ($CL_s$), estimated half life ($T_{1/2}$), elimination rate ($K_e$) and volume of distribution ($V_z$). Formation

clearance ($CL_f$) of 1'-hydroxymidazolam was calculated by dividing the amount in the urine (1'-hydroxymidazolam concentration in the urine times total urine volume) by the plasma midazolam $AUC_{0-T}$. One way repeated ANOVA with Bonferroni post hoc analysis was used to compare formation clearance in the four treatments. Significance was denoted by a p value $<0.05$.

**Population Pharmacokinetic Modeling.** The population pharmacokinetic model describing midazolam disposition under the conditions outlined in this study was constructed using a nonlinear mixed-effects approach as implemented in the NONMEM V1.1 program (Icon, Hanover MD). The population pharmacokinetics model consists of a pharmacokinetics structural model and a statistical model in which between subject and within subject variability are described. One and two-compartment structural pharmacokinetics models were investigated as base model structures. The one-compartment model structure was evaluated using the ADVAN 1 TRANS 2 routine and the two-compartment model structure was implemented as the ADVAN3 TRANS4 subroutine. The first order conditional estimation method (FOCE) with interaction was used to estimate all parameters. The inter-individual variability in the pharmacokinetic parameters was assumed to be log normally distributed. The residual variability was evaluated using three candidate model structures, additive, proportional and a combined additive and proportional model Yij=Fij*(1+Err(1))+Err(2).

The model building process was guided by analyzing the goodness of fit plots, precision of parameters estimates, and the objective function value (OFV) provided by NONMEM. After the base model was selected, we evaluated the effect of core temperature (TEM), heart rate (HR) and magnesium (MG) effect (Y/N) on the pharmacokinetic parameters (CL, V, or CL, $V_1$, $V_2$, Q). Covariate effects were evaluated using a forward stepwise addition and reverse deletion approach. The impact of the covariates were evaluated using the change in the $-2 \times \log$

likelihood (-2LL), visual diagnostics, successful minimization, parsimony and physiologic reasonableness of the covariate effects. Improvements in the model were accepted as significant ($p < 0.01$, 1d.f.) when a decrease of > 6.64 points in the OFV per added model parameter was observed. Diagnostic plots included population predicted concentrations (PRED) vs observed concentration, individual predicted concentrations (IPRE) vs observed concentration, population predicted concentrations (PRED) vs weighted residuals (WRES) and time vs weighted residuals (WRES). Empirical Bayes estimates for individual patient PK parameters clearances were generated and compared with the results from the non-compartmental analysis.

**Bootstrap Evaluation.** A nonparametric bootstrap approach using sampling with replacement was used to assess the robustness of the model estimates (Parke et al., 1999). Sampling with replacement involved creating a series of datasets of equal size to original data set that are generated by repeatedly sampling individuals from the original dataset, removing these individuals and replacing them at random. The model was refitted to each new data set and this process was repeated 1000 times. The stability of the final model was evaluated by examining the 95% confidence intervals (95% CI) of model parameter estimates. The Wings for NONMEM (G77 Fortran with WFN 408b) implementation for bootstrapping was utilized (Hayes et al.,1989).

**Simulation.** The population parameter estimates obtained from the final model were used to simulate the population average concentration-time profiles at three fixed core temperatures, 36.5°C, 34°C and 32°C, over a duration of 400 minutes. Midazolam two mg was administered in the simulation as iv three doses (as used in this study). The simulations were implemented in WinNonlin 4.01 use the population estimated parameters from the NONMEM.

## Results

**Subjects and Physiologic Variables.** Eight subjects provided informed consent and were screened for this study. Two subjects were excluded during screening due to laboratory values that were outside the normal range. The demographics and morphometrics of six subjects were: age 28.5 ± 7.6 (yr), height: 175.7± 4.7 (cm), mass: 78.8 ± 8.6 (kg), and body fat: 17.3 ± 3.2 (%). The procedure was well tolerated in all subjects. In our study, we observed relatively minimal sedation with ventilatory and cardiovascular functions unaffected by midazolam. No subject exceeded a value of '3' (Patient awake, responds to commands only) on the Ramsey sedation scale. Patient responds quickly to a voice command and no deeper sedation found. There was an effect of magnesium [B = 8.18 (1.66, 14.7), p = 0.014] and on heart rate. There was no effect of cold saline, magnesium, or time on respiratory rate and end-tidal CO2.

**Temperature.** Rapid infusion of 37ºC saline resulted in a small 0.4 ± 0.2ºC decrease from baseline. Infusion of 37ºC saline with magnesium resulted in a temperature reduction of 0.9 ± 0.3ºC. Infusion of cold saline reduced core temperature 1.4 ± 0.3ºC from baseline with a mean nadir temperature of 35.8°C ± 0.3°C. Addition of magnesium to the infusion reduced core temperature 1.8 ± 0.3ºC from baseline with the mean nadir at 35.4°C ± 0.4°C. In Cold and Cold+Mg groups, the duration (time below the 36.3°C which is the lowest value in the warm saline infusion groups) of the mild hypothermia is 47.0 ± 24.5min and 101.3 ± 26.6min, respectively. Statistically, there was an effect of cold saline [B = -0.48 (-0.87, -0.92) p = 0.015] and magnesium [B = -0.40 (-0.77, -0.02) p = 0.035] on core temperature. There was neither a time nor a cold saline/magnesium interaction on core temperature. Mean skin temperature changed over time [B = -0.007 (-0.01, 0.0) p = 0.004] but did not differ by the temperature infusion or the addition of magnesium. Cooling burden was calculated and a significant

difference was found between Warm and Cold+Mg groups (p=0.01). The core temperature curve and the cooling burden were shown in Figure 1.

**Midazolam Time-Concentration Profile and Noncompartmental Analysis.** Six individual time-plasma concentration profiles of midazolam of all four treatments were shown in Figure 2. The estimated $C_{max}$, $AUC_{0\text{-}inf}$, $CL_s$, $CL_f$ , $T_{1/2}$, $K_e$ and $V_z$ of the four treatments from noncompartmetnal analysis was shown in Table 1. A significant decrease in the 1'-hydroxymidazolam formation clearance was observed during Cold+Mg compared with Warm group (2.43 ± 0.782 mL/min/kg *vs* 3.41 ± 0.735 mL/min/kg, p=0.0168). Systemic clearance during Cold+Mg compared with Warm saline group demonstrated a trend towards a significant reduction during hypothermia vs normothermia (3.76 ± 0.386 mL/min/kg *vs* 4.49 ± 0.560 mL/min/kg, p=0.0568). No significant difference in $C_{max}$, $T_{1/2}$, $AUC_{0\text{-}inf,}$ and $V_z$ among the four treatments. Individual 1'-hydroxymidazolam formation clearance and midazoalm systemic clearance of four treatments were plotted in Figure 3.

**Population Based Nonlinear-Mixed Effect Pharmacokinetic Modeling.** The data set comprised 258 plasma concentrations from 24 visits. A two-compartment base model structure was significantly better at describing the disposition of midazolam than the one compartment model (OFV difference 16.0 points, 2df, p<0.001). The final two-compartment model including covariate relationships is shown below: CLs (L/hr) =18.5*(TEM/36.5)**4.24, $V_1$ (L)=9.11, Q (L/hr) =230*(TEM/36.5)**(-17.9), $V_2$ (L) =37.3+(HR/68)**(-5.08)+MG*(-3.75).

The final model had an objective function value (OFV) of 1152.0, 28.9U lower than the best base model OFV of 1180.9. Graphical model performance is depicted in Figure 5. Both systemic clearance and intercompartment clearance of midazolam were affected by core temperature. Temperature was not a significant covariate when tested in relation to the $V_1$ and $V_2$ parameters. Heart rate and magnesium significantly affected $V_2$. The presence of magnesium reduced the

volume of distribution by 3.75L (close to 10% volume of $V_2$). The $V_2$ changed 73.13% and 75.06% from the maximum to the minimum observed heart rate with or without magnesium, respectively. The pharmacokinetic parameters estimated from the NONMEM analysis were consistent with the non-compartmental analysis. Based on this model, the lowest core temperature 34.8°C in this study would result in a $CL_s$ of 16.0 L/hr which was 29.6% lower than the clearance from the highest core temperature 37.8°C 22.7L/hr. As temperature decreases, midazolam elimination from the central compartment declines. The model describes an estimated 11.1% reduction in midazolam clearance for every one degree reduction in core temperature from 36.5°C. The individual level model predicted relationships (based on the empirical Bayes estimates) between specific temperature and midazolam clearance are shown in Figure 5.

**Bootstrapping and Simulation.** The final model has been assessed further by nonparametric bootstrapping method, the results of bootstrap estimates were consistent with NONMEM estimates which means the final model was relatively stable and robust. NONMEM estimates and the 95% CI from bootstrapping are shown in Table 2. A concentration-time profile of midazolam simulated from the population level NONMEM parameters is shown in Figure 6. The simulation curve for a core temperature 32 °C has the highest AUC and $C_{max}$ followed by 34 °C and 36.5 °C. The simulations reflect the model predicted reductions in midazolam clearance at 32°C (a 42.8% reduction compared to 36.5°C) and 34°C (a 26.0% reduction compared to 36.5°C).

## Discussion

This study demonstrated that midazolam metabolism is reduced by mild and short duration of hypothermia in normal healthy volunteers. The results provided the basis for predicting alterations in midazolam clearance and possible drug therapy interaction involved of other CYP3A substrates under mild hypothermic conditions. There are two major observations from this study. First, the cold saline 30mL/kg infusion with midazolam and magnesium reduced the core temperature in normal healthy volunteers in the absence of anesthesia, however, the shivering was minimally suppressed in healthy subjects. Second, the clearance of midazolam was significantly affected by body temperature change in human from both non-compartmental pharmacokinetic analysis and population pharmacokinetic modeling. This model predicted that midazolam clearance would decrease 11.1% for each degree lower in core temperature from 36.5°C.

The body temperature changes observed in this study from both normothermia and hypothermia groups were consistent with previous studies. In our normothermia group, warm saline infusion with midazolam resulted in a small 0.4 ± 0.2ºC decrease from baseline. Addition of magnesium resulted in a temperature reduction of 0.9 ± 0.3ºC. Kurz et al. used midazolam and surface cooling to induce hypothermia and showed that midazolam impairs thermoregulatory control by decreasing sweating threshold 0.3 ºC, and decreasing shivering threshold around 0.6ºC (Kurz et al., 2005). Our previous study used just rapid warm saline infusion resulted in 0.5 ºC temperature reduction (Moore et al., 2008). In our hypothermia group, the lowest core temperature 35.4°C ± 0.4°C was observed in the cold saline infusion with magnesium group, co-infusion of 4g of magnesium during a cold saline infusion resulted in an additional 0.5ºC of cooling compared with cold saline infusion itself. We have previously reported that rapid

infusion of cold saline can effectively reduce body temperature in normal healthy volunteers compared with surface cooling (Moore et al., 2008; Hostler et al., 2009). Magnesium sulfate facilitates cooling through its known vasodilatory effects to promote peripheral heat exchange and blunt the shiver response upon cold saline infusion. Magnesium sulfate in total given dose of 8.75 to 16.75 g has been shown to increase the rate of hypothermia and improve the comfort of healthy subject during hypothermia induction (Zweifler et al., 2004). Previous studies have shown magnesium sulfate significantly reduce the shivering threshold (36.3 ±0.4 vs 36.6 ±0.3° C, p=0.04) in healthy volunteers which was consistent with the observation of this study (Wadhwa et al., 2005). In addition, magnesium may provide protection against ischemia in reducing CA1 neuronal death combined with modest hypothermia (35°C) in animal model (Zhu et al., 2005). Therefore, magnesium use in hypothermia deserves further study. Although the gain of temperature changes was small in this study, the infusion was well tolerated in this population and may prove of some benefit in achieving a target temperature in conscious patients. These results suggested that it is possible to reduce body temperature in conscious subjects, however cold saline infusion with midazolam and magnesium combination used in this study can only created an mild and short duration of hypothermia and did not sufficiently maintain hypothermia to a clinical desired level.

In this study, we evaluated the model compound midazolam as an index of CYP3A4/5 metabolism. Midazolam is a well known CYP3A probe mostly because it exclusively metabolized by human CYP3A4/5 isoforms, the metabolism is not flow dependent (low to medium extraction ratio), and midazolam is not a P-glycoprotein transporter substrate. The primary midazolam metabolite produced through CYP3A in human is 1'-hydroxymidazolam (~70%). Minor metabolites formed by CYP3A4 are 4'-hydroxymidazolam and 1',4'-dihydroxymidazolam (Galetin et al., 2005). Our results demonstrated that even in mild and short

duration of hypothermia, the 1'-hydroxymidazolam formation clearance in the Cold+Mg group was significant lower compared to normothermia. These results were consistent with previous preclinical and clinical studies. CYP3A activity during cooling was previously determined by ethylmorphine-N- demethylation *in vitro* in piglet liver microsomes. This study demonstrated a strong temperature dependence with CYP3A activity ($p<0.01$). Furthermore, this study indicated that the plasma concentration of fentanyl, another CYP3A substrate (liver blood flow dependent), significantly increased during hypothermia (31.6 ± 0.2 °C) for 6 hours and after rewarming ($p<0.001$ and $p<0.05$ respectively) (Fritz et al., 2005). The study by Fukuoka et al reported significant changes of midazolam clearance during moderate (32-34°C) hypothermia in eight brain injured patients who were given a continuous infusion of midazolam (Fukuoka et al., 2004). Collectively, previous preclinical or clinical studies have indicated the possible changes of CYP3A activity in cooling, however, the relationship of body temperature and CYP3A activity in human was still not clearly elucidated.

Our data analysis comprehensively estimated the pharmacokinetic parameters of midazolam during mild hypothermia in human and predicted the relationship between core temperature and CYP3A activity by using both noncompartmental pharmacokinetic analysis and population based nonlinear mixed effect pharmacokinetic modeling. The noncompartmental pharmacokinetic analysis assumed that the clearance was constant over time within group which may not be a sufficient and sensitive estimate for the changes of systemic clearance with changing temperature. Our study was designed to record core temperature and midazolam concentration continuously over time, therefore, allowing the time varying covariate of temperature to be modeled using a nonlinear mixed-effects population pharmacokinetic approach. The approach provides for the preservation of data structure so that each individual condition contributes to the population description. The model estimate of clearance of midazolam was 18.5 L/hr at 36.5 °C

which was consistent with previous studies (de Wildt et al., 2003; Shimizu et al., 2007; Bolon et al., 2003). The relationship between core body temperature and the midazolam systemic clearance was described using the following function: CL (L/hr)=18.5* (TEM/36.5)**4.24, which provided the basis for predicting the temperature relationship with clearance of midazolam. This population model predicts that a 1°C reduction in core temperature from 36.5°C produces an 11.1% reduction in midazolam clearance. This 11.1% percentage reduction per degree change in core temperature is consistent with the results of Leon et al who reported that for each 1°C decrease in temperature, a 10% reduction in tissue metabolic requirements and free radical production (Leon 2004). In addition, the study by Caldwell et al reported that 11.3% clearance of vecuronium decreased with per degree (Celsius) temperature change in healthy human volunteers (Caldwell et al., 2000). A study by Michelsen et al also demonstrated that the clearance of remifentanil decreased by 6.37% for each degree (Celsius) below 37°C in patients who undergoing coronary artery bypass surgery (Michelsen et al., 2001). Our pharmacokinetic model predicted a midazolam disposition changed under mild hypothermia condition was consistent with previous studies. The model suggested a predictive decrease in drug metabolism per degree change in body temperature, and the predictive utility of these estimates in a prospective validation cohort remains to be determined.

Due to the shivering response and discomfort of healthy human subjects, the cooling period of this study was designed for duration of 200 minutes followed by rewarming. Due to the likelihood of alterations in clearance upon rewarming, blood samples were only drawn during the period of time when subjects were actively on the cooling protocol, which was from time zero to 200 minutes. This duration may not be long enough to capture a better midazolam disposition curve, however it was not possible to collect samples at a later time point while accurately estimating the cooling effect on the metabolism of midazolam in the hypothermia healthy human

study. In addition, the estimated half life of midazolam in healthy normothermia control group of this study was consistent with literature as previously mentioned.

Future studies to determine specific dosing guidelines for commonly used medications in critically ill patients during cooling are needed to prevent the potential therapy-drug interactions in this highly susceptible patient population. The magnitude of pathway specific alterations in drug elimination continue needed to be elucidated in order to begin to develop for specific dosing recommendations. Until such guidelines are developed, vigilance with drug response monitoring is a clinical necessity.

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Footnotes:

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**Fig.1.** Core temperature (Mean ±SD) over time curves of four treatment groups. The temperatures were recorded every 2 minutes during the first 30 minutes and every 10 minutes thereafter. Cold burden area was calculated from each individual temperature time curve. There was a significant difference between Warm and Cold+Mg groups (p=0.01) of cold burden area. (■Warm, ▲Warm+Mg, ○ Cold, ◊ Cold+Mg)

**Fig. 2.** Individual midazolam time –plasma concentration profile (each individual has four curves from four treatments) and six subjects time-concentration curves (Mean ±SD). (■Warm, ▲Warm+Mg, ○ Cold, ◊ Cold+Mg)

**Fig. 3.** Four group 1'-hydroxymidazolam formation clearance and midazolam systemic clearance from non-compartmental analysis estimation with Mean bar. Difference was observed in 1'-hydroxymidazolam formation clearance between Warm and Cold+Mg (p=0.0168). A tread toward significant in systemic clearance within four treatment groups (p=0.0568). (■Warm, ▲Warm+Mg, ○ Cold, ◊ Cold+Mg)

**Fig. 4.** Model diagnostic plots. Goodness-of-fit plots for the population pharmacokinetic model from NONMEM analysis. Individual predicted concentrations vs observed concentrations, and the predicted concentrations vs observed concentrations. The straight lines are the lines of unity (A-B). Population predicted concentrations vs weighted residual and Time vs weighted residual (C-D).

**Fig. 5.** The relationship between core body temperature ( from the highest body temperature 37.8°C to the lowest body temperature 34.8 °C observed in this study) and individual midazolam systemic clearance estimated from the final population pharmacokinetic model.

**Fig. 6.** The simulated time- concentration profiles of midazolam in core temperature 32°C, 34°C and 36.5°C. The curves were generated from Winonlin software based on the population pharmacokinetic parameters estimated from NONMEM. The simulation curve for a core temperature 32 °C (* symbols) has the highest AUC and $C_{max}$ followed by 34 °C (♦ symbols) and 36.5 °C (■ symbols) . The simulations reflect the model predicted reductions in midazolam clearance at 32°C (a 42.8% reduction compared to 36.5°C) and 34°C (a 26.0% reduction compared to 36.5°C).

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TABLE 1. Pharmacokinetic variables of four treatments from non-compartmental analysis. One way repeated ANOVA has been used to detect the difference among the four treatment groups.

| | **Warm** | **Warm/Mg** | **Cold** | **Cold/Mg** | **P value** |
|---|---|---|---|---|---|
| $C_{max}$ (ng/mL) | 123 (23.1) | 120 (6.88) | 116 (23.0) | 132 (16.2) | 0.360 |
| $T_{1/2}$ (min) | 107 (20.8) | 107 ( 20.2) | 119 (29.4) | 96.3 (9.50) | 0.212 |
| $AUC_{0\text{-}T}$ (ng*min/mL) | 13847 (1644) | 15069 (1160) | 15977 (2897) | 17069 (2268) | 0.0364* |
| $AUC_{0\text{-}inf}$ (ng*min/mL) | 17110 (1634) | 18679 (788) | 20760 (4332) | 20578 (2333) | 0.0649 |
| $V_z$ (mL) | 54495 (11663) | 49762 (9594) | 50281(10632) | 41364 (7113) | 0.0908 |
| $Cl_s$ (mL/min/kg) | 4.49 (0.560) | 4.12 (0.492) | 3.83 (0.881) | 3.76 (0.386) | 0.0568 |
| $Cl_f$ (mL/min/kg) | 3.41 (0.735) | 2.65 (0.923) | 2.56 (0.917) | 2.43 (0.782) | 0.0168* |

*Data presented as mean (SD). *p < 0.05*

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TABLE 2. NONMEM and Bootstrap estimates with 95% confidence interval.

| | NONMEM Estimates | Bootstrap Mean (95% CI) |
|---|---|---|
| THETA(1) | 18.5 | 18.4 ( 18.36-18.44) |
| THETA(2) | 9.11 | 9.68 ( 9.39~9.98) |
| THETA(3) | 230 | 253 (202~304) |
| THETA(4) | 37.3 | 36.7 ( 36.5~37.0) |
| THETA(5) | 4.24 | 4.11 ( 3.86~4.37) |
| THETA(6) | -17.9 | -22.1 (-23.3~-20.8) |
| THETA(7) | -5.08 | -4.68 ( -4.81~ -4.55) |
| THETA(8) | -3.75 | -4.12 ( -4.21~ -4.03) |
| ETA(1) | 0.0762 | 0.0618 ( 0.059~0.064) |
| ETA(2) | 0.0543 | 0.487 (0.466~0.509) |
| ETA(3) | 0.135 | 0.100 (0.097, 0.103) |
| ERR(1) | 0.166 | 0.169 (0.168, 0.171) |
| ERR(2) | 0.0992 | 1.64 (1.53, 1.75) |

*NONMEM modeling control stream: TVCL=THETA(1)*(TEM/36.5)**THETA(5);*
*CL = TVCL*EXP(ETA(1)); TVV1=THETA(2); V1 = TVV1*EXP(ETA(2));*
*TVQ = THETA(3)*(TEM/36.5)**THETA(6); Q = TVQ;*
*TVV2 = THETA(4)+(HR/68)**THETA(7)+MG*THETA(8); V2=TVV2*EXP(ETA(3));*
*S1 = V1, Y=F+F*ERR(1)+ERR(2);*

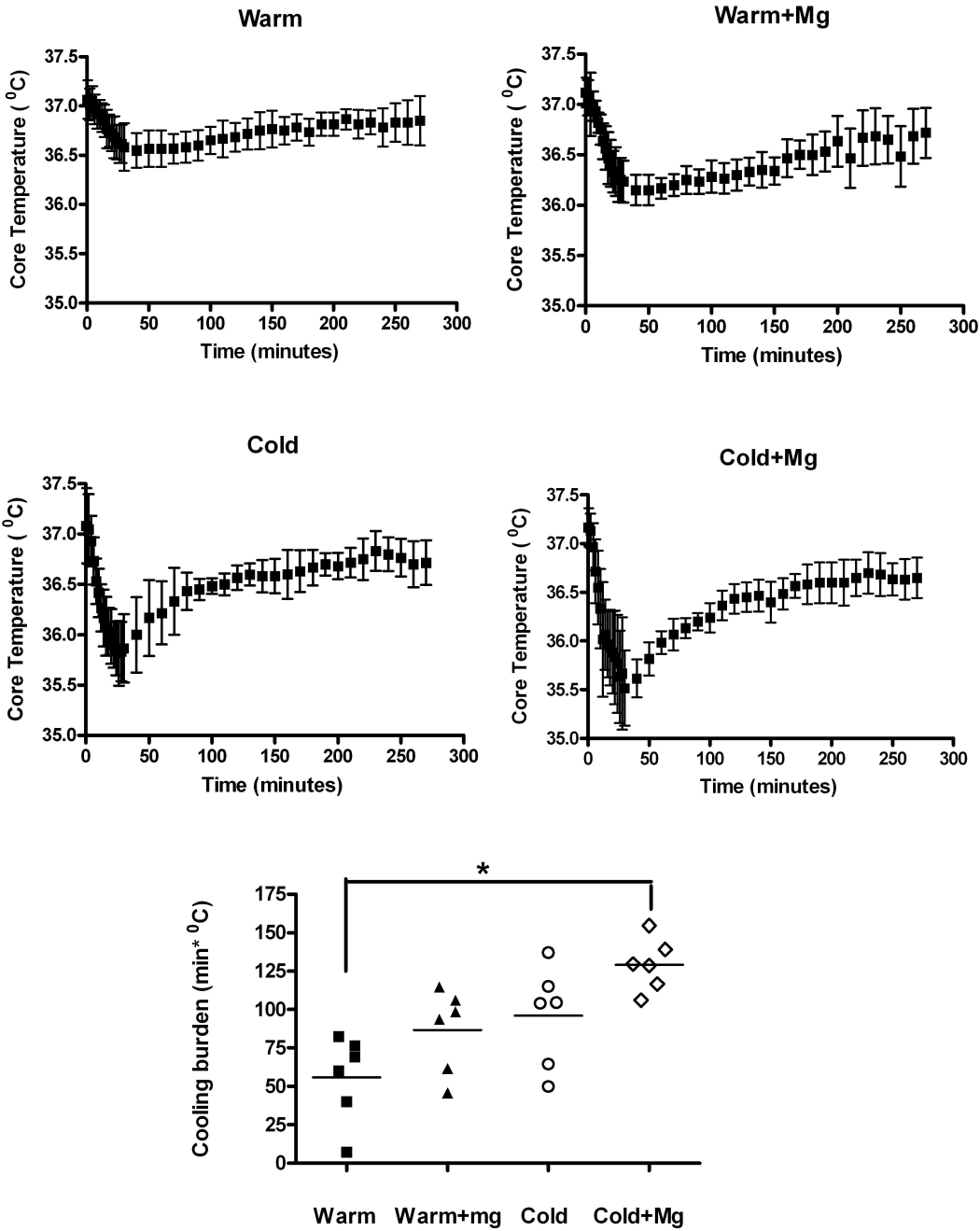

Warm
Warm+Mg
Cold
Cold+Mg
Core Temperature ( $^0$C)
Time (minutes)
37.5
37.0
36.5
36.0
35.5
35.0
0
50
100
150
200
250
300
Cooling burden (min* $^0$C)
*
175
150
125
100
75
50
25
0
Warm
Warm+mg
Cold
Cold+Mg

**Figure 1**

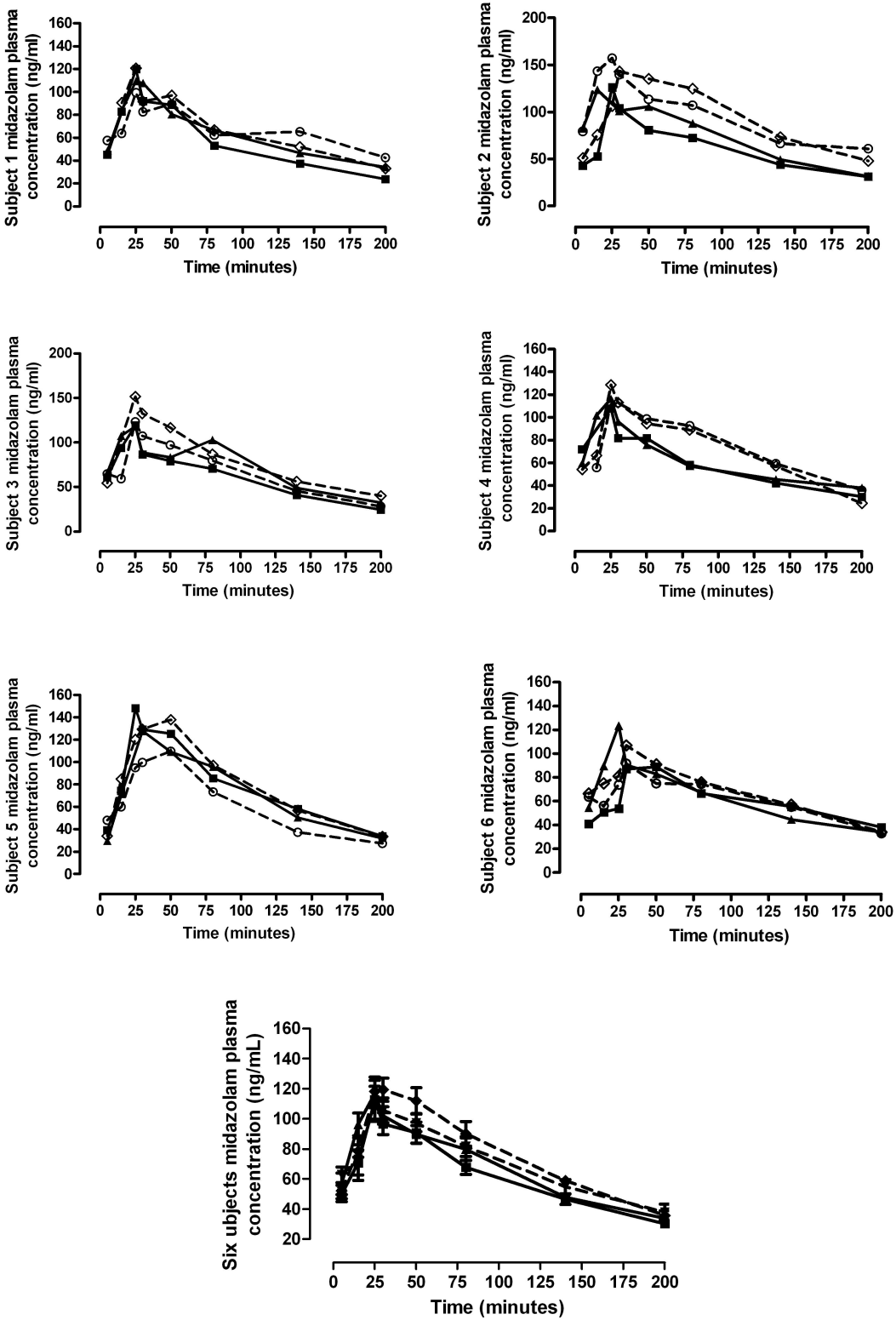
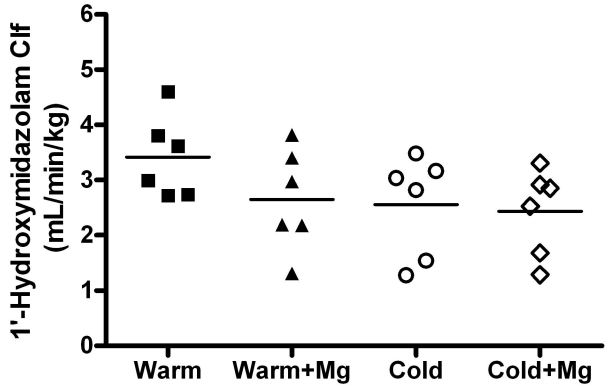

Subject 1 midazolam plasma concentration (ng/ml)
Time (minutes)
Subject 2 midazolam plasma concentration (ng/ml)
Time (minutes)
Subject 3 midazolam plasma concentration (ng/ml)
Time (minutes)
Subject 4 midazolam plasma concentration (ng/ml)
Time (minutes)
Subject 5 midazolam plasma concentration (ng/ml)
Time (minutes)
Subject 6 midazolam plasma concentration (ng/ml)
Time (minutes)
Six ubjects midazolam plasma concentration (ng/mL)
Time (minutes)

**Figure 2**


1'-Hydroxymidazolam Clf
(mL/min/kg)
6
5
4
3
2
1
0
Warm
Warm+Mg
Cold
Cold+Mg

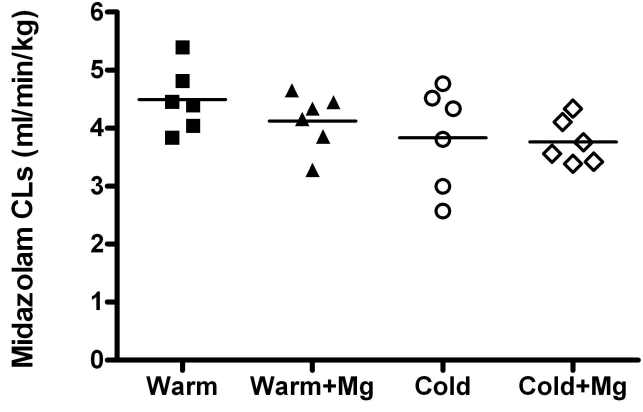

Midazolam CLs (ml/min/kg)
6
5
4
3
2
1
0
Warm
Warm+Mg
Cold
Cold+Mg

Figure 3

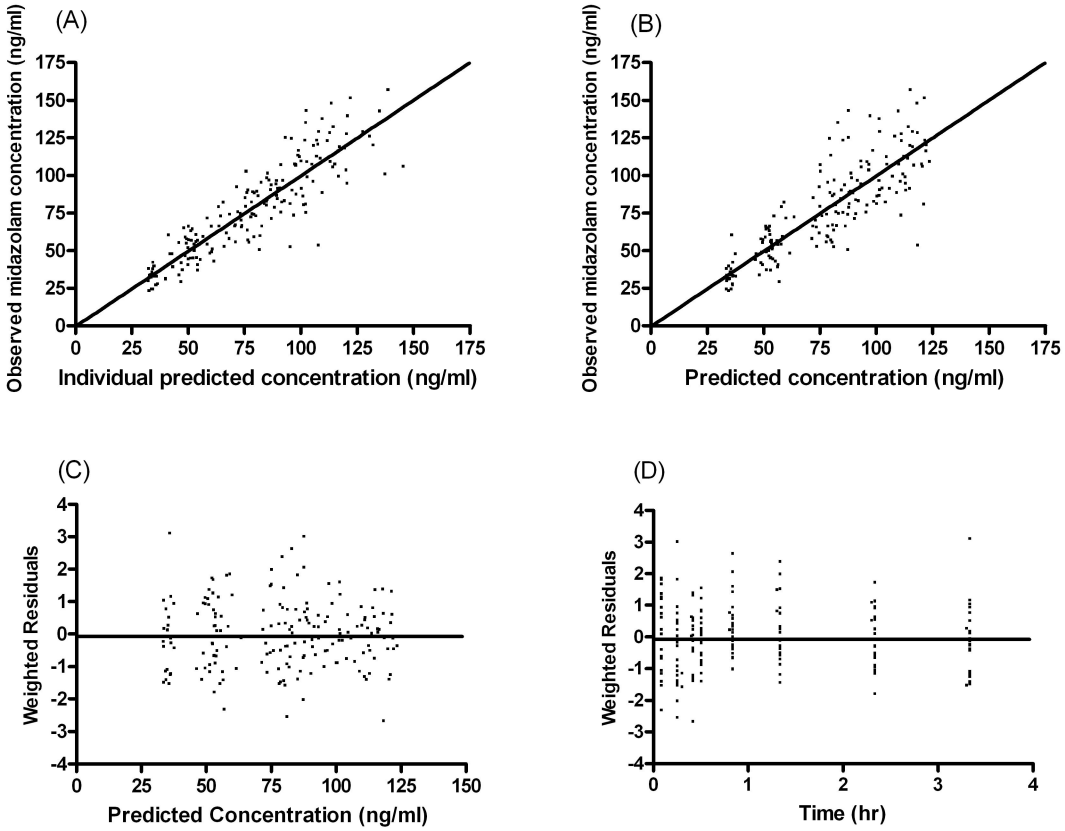

(A)
Observed midazolam concentration (ng/ml)
Individual predicted concentration (ng/ml)
(B)
Observed midazolam concentration (ng/ml)
Predicted concentration (ng/ml)
(C)
Weighted Residuals
Predicted Concentration (ng/ml)
(D)
Weighted Residuals
Time (hr)

**Figure 4**

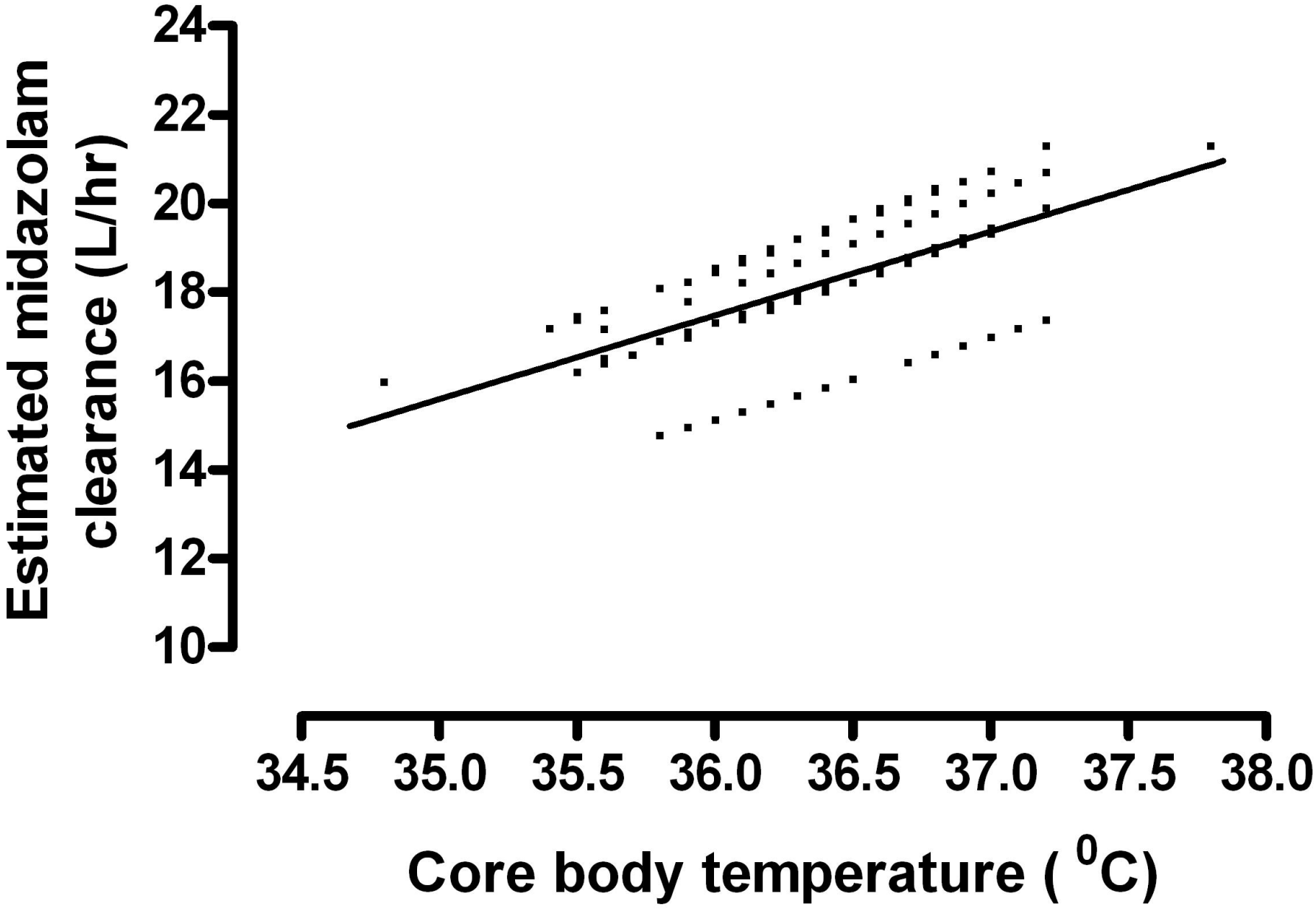

Estimated midazolam clearance (L/hr)
24
22
20
18
16
14
12
10
34.5
35.0
35.5
36.0
36.5
37.0
37.5
38.0
Core body temperature ( 0C)

Figure 5

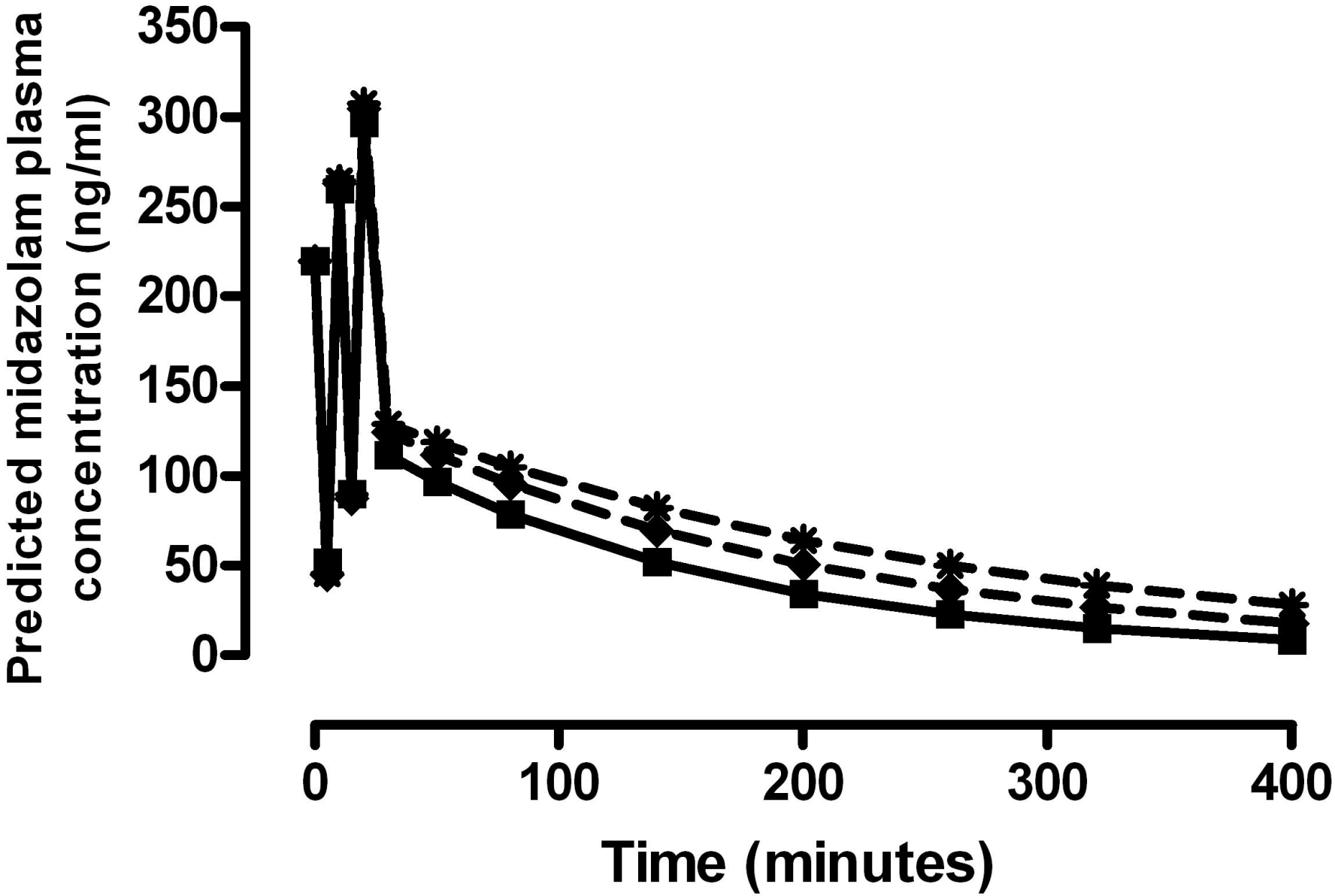

Predicted midazolam plasma concentration (ng/ml)
350
300
250
200
150
100
50
0
0
100
200
300
400
Time (minutes)

Figure 6