Title

Verification of a Maternal-Fetal Physiologically Based Pharmacokinetic Model for Passive Placental Permeability Drugs

Zufei Zhang and Jashvant D. Unadkat

Department of Pharmaceutics, University of Washington, Seattle, WA

DMD Fast Forward. Published on January 3, 2017 as DOI: 10.1124/dmd.116.073957
This article has not been copyedited and formatted. The final version may differ from this version.

Running Title: Verification of a maternal-fetal PBPK model

Corresponding Author:

Dr. Jashvant D. Unadkat

Department of Pharmaceutics

University of Washington

Box 357610

Seattle, WA 98195

Telephone: 206-543-9434

Fax: 206-543-3204

E-mail: jash@u.washington.edu

Number of words in the Abstract: 244

Number of words in the Introduction: 421

Number of words in the Discussion: 1768

Number of text pages: 22

Number of tables: 2

Number of figures: 4

Number of references: 66

Abbreviations:

ADME: absorption, distribution, metabolism and excretion; AUC: area under the curve; BCRP: Breast cancer resistance protein; B/P: blood to plasma concentration ratio; BCS: Biopharmaceutics Classification System; CL_{iv}: intravenous clearance; $CL_{int,u}$: unbound intrinsic clearance; CL_{PD}: transplacental passive diffusion clearance; $CL_{PD,u}$: unbound transplacental passive diffusion clearance; CL_r: renal clearance; C_{max}: maximum plasma concentration; CYP: cytochrome P450; C-T: concentration-time; CV: coefficient of variation; ENT: equilibratuve nucleoside transporter; $f_{m,CYP}$: fraction metabolized by a given CYP enzyme; $f_{u,p}$: fraction unbound in plasma; F_a: fraction absorbed; F_g: fraction escaping gut metabolism; F_h: hepatic bioavailability; GW: gestational week; HLM, human liver microsomes; k_a: first order absorption rate constant; K_p: tissue-to-plasma partition coefficient; MRP5: Multidrug resistance-associated protein 5; OATs: Organic anion transporters; P_{app}: apparent membrane permeability; PBPK model: physiologically-based pharmacokinetic model; P-gp: P-glycoprotein; PK: pharmacokinetics; RSS: residual sum of squares; UGT: UDP-glucuronosyltransferase; T_1, T_2 and T_3: 1^{st}, 2^{nd} and 3^{rd} trimester; V_{ss}: volume of distribution at steady state.

Abstract:

Fetal exposure to drugs cannot be readily estimated from single time point cord blood sampling at the time of delivery. Therefore, we developed a physiologically-based pharmacokinetic (PBPK) model to estimate fetal drug exposure throughout pregnancy. Here we report verification of this novel maternal-fetal physiologically-based pharmacokinetic (m-f-PBPK) model for drugs that passively diffuse across the placenta and are not metabolized/transported there. Our recently built m-f-PBPK model was populated with gestational age-dependent changes in maternal drug disposition and maternal-fetal physiology. Using midazolam as an *in vivo* calibrator, the transplacental passive diffusion clearance of theophylline and zidovudine was first estimated. Then, for verification, the predicted maternal plasma (MP) and umbilical venous (UV) plasma drug concentrations by our m-f-PBPK were compared against those observed at term. Overall, our m-f-PBPK model well predicted the maternal and fetal exposure to the two verification drugs, theophylline and zidovudine, at term, across a range of dosing regimens, with nearly all observed MP and UV plasma drug concentrations falling within the 90% prediction interval [i.e.5^{th} -95^{th} percentile range of a virtual pregnant population (n=100)]. Prediction precision and bias of theophylline MP and UV were 14.5% and 12.4%, and 9.4% and 7.5%, respectively. Further, for zidovudine, after the exclusion of one unexpectedly low MP concentration, prediction precision and bias for MP and UV were 50.3 % and 30.2, and 28.3% and 15.0%, respectively. This m-f-PBPK should be useful to predict fetal exposure to drugs, throughout pregnancy, for drugs that passively diffuse across the placenta.

Introduction

Medication use during pregnancy is remarkably common. The average number of over-the-counter and prescription drugs used by women during pregnancy increased from 2.5 in 1976-78 to 4.2 in 2006-08 in the United States alone (Mitchell et al., 2011). The same study also revealed that, over the same period, use of such drugs during the first trimester increased from 1.6 to 2.6. The above statistics are not surprising as pregnant women need to be treated for many medical conditions, either pre-exiting (e.g. epilepsy, asthma, HIV-infection) or pregnancy-induced (e.g. gestational hypertension, diabetes, preeclampsia). In addition, the fetus is sometimes the therapeutic target, for example, to prevent vertical transmission of HIV (e.g. zidovudine, lamivudine), to treat fetal supraventricular tachycardia (e.g. digoxin) or to prevent fetal respiratory distress syndrome (e.g. dexamethasone) (Evans et al., 1993).

Regardless of whether or not the fetus is the intended target of pharmacotherapy during pregnancy, the fetus is *de facto* exposed to all drugs taken by the mother. Clearly, quantitative assessment of fetal exposure to drugs, especially during early gestation when the fetus is more prone to teratogenic effects (Chung, 2004), is important from both efficacy and toxicity standpoint. However, fetal drug exposure cannot be ethically studied before the time of delivery, when a single time cord blood sample [usually umbilical venous (UV) plasma] can be safely obtained. As we have shown before (manuscript submitted), this UV plasma concentration, except at steady-state after maternal drug infusion, does not provide information on fetal drug exposure, a determinant of drug efficacy and/or toxicity. Furthermore, these term or near-term data cannot be readily extrapolated to early gestation. Therefore, there is a pressing need to

develop other *in silico* methods to predict fetal drug exposure, such as **p**hysiologically-**b**ased **p**harmaco**k**inetic (PBPK) modeling and simulation approaches.

Recently, we expanded our previously verified maternal PBPK (m-PBPK) model to include the fetus (m-f-PBPK model) (manuscript submitted). The placenta, amniotic fluid, and fetal organs important for drug disposition as well as the gestational age-dependent fetal physiological changes (when available) were included in this m-f-PBPK model. Once developed, all models need to be verified. However, in this case, the only data available for verification are the UV and maternal plasma (MP) drug concentrations, often obtained simultaneously, at the time of birth. Therefore, as a first step, the primary objective of this study was to verify our novel m-f-PBPK model for drugs that cross the placenta predominantly by passive diffusion and for which UV and MP concentrations at the time of delivery are available in the literature (i.e. theophylline, and zidovudine).

Materials and Methods

The General Maternal-Fetal PBPK Model Structure and Key Assumptions

The general m-f-PBPK structure (**Supplementary Figure S1b**) and the key assumptions made in constructing the model have been described in detail (manuscript submitted). In brief, a fetal PBPK model was developed to replace the lumped, non-eliminating placental-fetal unit in our verified m-PBPK model (Ke et al., 2012; Ke et al., 2013a; Ke et al., 2013b). The resulting m-f-PBPK contained organs that are relevant to fetal disposition of pharmaceutical agents, including those involved in drug disposition (e.g. fetal liver, fetal kidneys) and in drug efficacy/toxicity (e.g. fetal brain). The model also contained compartments representing the placenta and the amniotic fluid. The ordinary differential equations defining mass balance in the maternal PBPK have been described

previously (Gaohua et al., 2012; Ke et al., 2012), whereas those describing the fetal PBPK are provided in supplementary information (**Supplementary Methods**). Briefly, all tissues except the placenta were regarded as well-stirred tissues; that is, the unbound tissue drug concentration is in instant equilibrium with the unbound drug concentration in the emergent venous blood. The placenta was modeled as a permeability-limited tissue and therefore it was subdivided into maternal placental blood, placenta tissue, and fetal placental blood compartments. Only the unbound, unionized fraction of drug can passively diffuse across the placenta. The bidirectional unbound maternal-placental and fetal-placental transplacental passive diffusion clearances ($CL_{PD,u}$) across the placenta were assumed to be equal (Tuntland et al., 1999). Our model has the capability of including placental transport and fetoplacental metabolism of drugs when quantitative data on transporters and metabolic enzymes become available. Since this fetal PBPK does not contain an umbilical vein compartment *per se*, the predicted fetal plasma drug concentration in the central venous blood compartment was assumed to represent that in umbilical vein.

Known gestational age-dependent changes in maternal and fetal physiology (e.g. blood flows, organ volumes, etc.) from recent literature meta-analyses were incorporated into the m-f-PBPK (Abduljalil et al., 2012) (manuscript submitted). The change in drug unbound fraction in plasma ($f_{u,p}$) was assumed to result from altered serum albumin concentration during pregnancy and was accounted for as described previously (Ke et al., 2012). Maternal hepatic 3A and 1A2 activity was assumed to increase by 99% [as measured by 1'-hydroxymidazolam formation clearance (Hebert et al., 2008)] and decrease by 65% [as indicated by salivary caffeine clearance (Tracy et al., 2005)] during

the third trimester (T3), respectively]. Current clinical data suggest that maternal UGT2B7 activity is unchanged during pregnancy (Anderson, 2005; Tasnif et al., 2016). Therefore, we assumed that maternal hepatic or extrahepatic UGT2B7 activity does not alter during pregnancy. Maternal renal glomerular filtration rate was assumed to increase by 33% at term [gestational week (GW) 40] (Abduljalil et al., 2012). For zidovudine, its renal net secretion clearance in the mother was assumed to be unaltered during pregnancy.

General Workflow of PBPK Model Development and Model Verification Criterion

Drug-specific parameters of midazolam and theophylline in non-pregnant subjects (**Table 1**) were obtained from our previous publications to populate their respective m-f-PBPK models (Ke et al., 2012; Ke et al., 2013a), whereas those of zidovudine were first refined based on the Simcyp Simulator® Version 14 (Simcyp Ltd., A Certara Company, Sheffield, UK) compound library using our previously published approach (Ke et al., 2013b). Briefly, refinements of zidovudine drug-specific parameters were made if the predicted zidovudine population mean plasma C-T curve in non-pregnant population using our MATLAB® 13-compartment PBPK model (**Supplementary Figure S1a**) significantly deviated from that observed. The refined zidovudine PK parameters were subsequently used to populate the zidovudine m-f-PBPK model. In addition, the available interindividual variability in physiological parameters and in drug-specific absorption, distribution, metabolism and elimination (ADME) processes in healthy volunteers (for zidovudine) or in the pregnant women (for midazolam, theophylline, and zidovudine at GW 0) was predicted within the Simcyp Simulator®. In brief, a compound profile was first constructed for each of these drugs within the Simcyp Simulator® using their respective drug-specific parameters. Interindiviudal variabilities associated with these

DMD Fast Forward. Published on January 3, 2017 as DOI: 10.1124/dmd.116.073957
This article has not been copyedited and formatted. The final version may differ from this version.

parameters were those specified by the Simcyp Simulator®. Then, the above predicted ADME characteristics for each virtual individual was used for the simulations conducted by the MATLAB® 13-compartment PBPK model (for zidovudine only) or by the m-f-PBPK model (for midazolam, theophylline, and zidovudine). For the pregnant population, the reported gestational age-dependent changes in maternal and fetal physiology (Abduljalil et al., 2012)(manuscript submitted) and the changes in maternal hepatic enzyme activity based on phenotyping studies conducted in pregnant women were taken into account as described above.

For each of the above test compounds, MP and UV drug C-T profiles were simulated in a virtual population consisting of 100 pregnant women at GW 40. The model was deemed to have met our verification criterion if the observed individual UV and MP drug concentrations (except midazolam MP concentrations, where the mean values from 8 subjects were used) at the time of delivery (extracted or digitized from literature using MATLAB® Grabit m.file ; available free online at http://www.mathworks.com/matlabcentral) fell within the 90% prediction interval (5^{th} - 95^{th} percentile range of the virtual population) calculated based on the interindividual variability in the maternal PK. Additionally, model prediction precision and prediction bias were evaluated by calculating the mean absolute prediction error ($\overline{APE}$; calculated as $\frac{\sum_{i=1}^{n} \frac{|Pred_i - Obs_i|}{Obs_i}}{n}$) and the mean prediction error ($\overline{PE}$; calculated as $\frac{\sum_{i=1}^{n} \frac{Pred_i - Obs_i}{Obs_i}}{n}$), where Pred and Obs denote the predicted and observed values, respectively (Sheiner and Beal, 1985).

DMD Fast Forward. Published on January 3, 2017 as DOI: 10.1124/dmd.116.073957
This article has not been copyedited and formatted. The final version may differ from this version.

Estimation of in vivo Transplacental Passive Diffusion Clearance of Drugs

Because data on the *in vivo* transplacental passive diffusion clearance (CL_{PD}) of drugs are not available in the literature, we chose midazolam as an *in vivo* calibrator to estimate CL_{PD} of theophylline and zidovudine (the same approach can be used for any drug). First, we optimized the *in vivo* $CL_{PD,u}$ of midazolam (see below). Then, the $CL_{PD,u}$ of drug X (theophylline or zidovudine) was estimated by scaling $CL_{PD,u}$ of midazolam using **Eq. 1**.

$$CL_{PD,u,X} = \frac{\overline{P_{app,X}}}{\overline{P_{app,midazolam}}} \times CL_{PD,u,midazolam} \text{ (L/h) (\textbf{Eq. 1})}$$

where $CL_{PD,u,midazolam}$ is the optimized *in vivo* $CL_{PD,u}$ (L/h) of midazolam (see below), and $\overline{P_{app,X}}$ and $\overline{P_{app,MDZ}}$ are the mean P_{app} values ($nm \cdot s^{-1}$) of drug X and midazolam, respectively. Reported P_{app} values of model drugs were collected from multiple sources in the literature (**Table 2**). The average P_{app} values of midazolam, theophylline, and zidovudine used were obtained by computing the mean values of Papp reported by 5, 5, and 7 independent studies, respectively. For physiological relevance and to avoid the confounding factor of binding, we only included studies conducted in established epithelial cell lines that form tight junctions between cells in monolayer cultures (i.e. MDCK and Caco-2) in the absence of serum/binding proteins.

Midazolam m-f-PBPK Model

First, the predicted midazolam plasma C-T profile, following a single 15mg oral dose in thirteen pregnant women (GW=40) proceeding elective cesarean section surgery, was compared against the observed data (Kanto et al., 1983). Midazolam drug-specific parameters, previously validated, are outlined in **Table 1** (Ke et al., 2012). CYP3A metabolism occurs in maternal gut, liver, and fetal liver. The observed 99% increase in

DMD Fast Forward. Published on January 3, 2017 as DOI: 10.1124/dmd.116.073957
This article has not been copyedited and formatted. The final version may differ from this version.

CYP3A activity was confined to maternal liver as we have previously shown that only maternal hepatic CYP3A activity appears to be induced during pregnancy (Zhang et al., 2008; Ke et al., 2012). To match the observed midazolam maternal absorption profile, a lag time of 0.1 h was introduced and a first-order absorption rate constant k_a of 4.0 h^{-1} was chosen through sensitivity analysis (data not shown). Because fetal liver predominantly expresses CYP3A7 (Shuster et al., 2014), fetal hepatic intrinsic clearance of midazolam ($CL_{f,hep,int}$) was estimated using **Eq.2**.

$$CL_{f,hep,int} = \frac{V_{max,3A7} \times A_{CYP3A7} \times MPPGL \times W_{f,liver}}{K_{m,CYP3A7}} \quad \textbf{(Eq. 2)}$$

Where $V_{max,3A7}$ and $K_{m,CYP3A7}$ are the maximal velocity and Michaelis-Menten constant determined in recombinant CYP3A7, A_{CYP3A7} is CYP3A7 abundance per mg microsomal protein, MPPGL is the fetal hepatic microsomal protein concentration per gram fetal liver, and $W_{f,liver}$ is fetal liver weight at term. Reported *in vitro* V_{max} /K_m value of midazolam hydroxylation in recombinant CYP3A7 is 2.1 mL/h /nmol P450 (Williams et al., 2002) and it is estimated that fetal liver microsomes contain 0.3 nmol P450 proteins per mg protein (Barter et al., 2007). Fetal MMPGL is 26 mg/g liver (Barter et al., 2008) and the average fetal liver at term weighs ~130 g (Abduljalil et al., 2012). Therefore, fetal hepatic $CL_{f,hep,int,}$ mediated by CYP3A7 was estimated as 2.13 L/h. The $CL_{PD,u}$ value of midazolam was optimized through sensitivity analysis. Briefly, the magnitude of midazolam $CL_{PD,u}$ was varied to reduce the residual sum of squares between the predicated and observed fetal C-T profile [RSS; calculated as $\sum_{i=1}^{n}(y_{pred} - y_{obs})^2$,where y_{pred} and y_{obs} refer to the predicted and observed fetal plasma midazolam concentrations, respectively]. The observed fetal C-T profile was created by **pooling** the

reported midazolam UV plasma concentrations from seven newborns in the same study (Kanto et al., 1983).

Theophylline m-f-PBPK Model

Maternal theophylline drug-specific parameters were those previously published (**Table 1**) (Ke et al., 2013a). Because the major placental CYP1A isoform, CYP1A1, has negligible contribution to theophylline metabolism (Ha et al., 1995) and no report on CYP1A2 expression in the term placenta or fetal liver is available, the placenta and fetal liver were considered as non-metabolizing organs for theophylline. Using the estimated $CL_{PD,u,theophylline}$ (**Eq. 1**), maternal and fetal theophylline C-T profiles following multiple oral doses of theophylline were simulated and compared with the observed data using the verification criterion described above. The observed theophylline data were from a study where 10 asthmatic women with normal pregnancies were administered 160 mg theophylline (in the form of aminophylline) every 6 hours for 30 hours prior to delivery (Ron et al., 1984). The observed maternal and fetal C-T profiles were created by **pooling** single time point MP and UV plasma theophylline concentrations from 10 **maternal-fetal pairs**.

Zidovudine m-f-PBPK Model

In non-pregnant adults following an intravenous dose, only ~17% of zidovudine is excreted unchanged in the urine, whereas 67% of the intravenous dose is recovered in the urine as 5'-O-gluduronide via UGT2B7 mediated glucuronidation (Cload, 1989; Stagg et al., 1992). Other identified metabolites include its active triphosphate metabolite formed via sequential intracellular phosphorylation (Veal et al., 1994), 3'-amino-3'-deoxythymidine, and 3'-amino-3'-deoxythymidine glucuronide (Stagg et al., 1992).

Average zidovudine intravenous plasma clearance is 91 L/h normalized to 70 kg body weight (Collins and Unadkat, 1989). Thus, plasma zidovudine glucuronidation clearance is estimated as 61 L/h. These data, in conjunction with the observed zidovudine absolute bioavailability (F) of ~ 63% (Klecker et al., 1987), suggests considerable extra-hepatic zidovudine metabolism. However, although UGT2B7 is expressed in the gut, liver, and kidneys (Ohno and Nakajin, 2009), investigations on the extrahepatic metabolism of zidovudine revealed that zidovudine is not glucuronidated by gut microsomes and that renal glucuronidation is minimal (Cretton et al., 1991; Howe et al., 1992; Knights et al., 2016). Moreover, published *in vitro*-to-*in vivo* (IVIVE) approach based on human liver microsomal data substantially underpredicted zidovudine glucuronidation clearance by a factor of 30.5 (Kilford et al., 2009). Based on these analyses, we speculate that there are unidentified extrahepatic, non-renal pathways responsible for zidovudine metabolism (glucuronidation and non-glucuronidation) *in vivo*. Therefore, to recapitulate zidovudine intravenous clearance, zidovudine hepatic unbound intrinsic clearance ($CL_{hep,int,u}$) was calculated using the well-stirred liver model

$$CL_{hep,int,u} = \frac{CL_{hep,b}}{\frac{fu_p}{B/P} \times (1 - \frac{CL_{hep,b}}{Q_H})} \textbf{ (Eq. 3)}$$

Where fu_p is the fraction unbound in the plasma, $CL_{hep,b}$ is hepatic blood clearance $\left[\frac{CL_{iv}}{B/P} \cdot \left(1 - \frac{F}{F_a F_g}\right) = 24.94\ L/h\right]$, B/P is the blood to plasma concentration ratio, and Q_H is the hepatic blood flow (90 L/h). A systemic plasma metabolic clearance of 52.8 L/h ($CL_{iv} - CL_{hep} - CL_r$) was assigned to the unidentified extrahepatic zidovudine metabolic pathways. Using the above detailed PK parameters (**Table 1**), the predicted zidovudine plasma C-T curves using the 13-compartment PBPK model were compared

with those observed following one hour intravenous infusion of 2.5 mg/kg (**Figure 3a**) or two different single oral doses (200 and 300 mg) in non-pregnant asymptomatic HIV-infected male patients with normal hepatic and renal functions (**Figure 3b** and **Figure 3c**, respectively) (Cload, 1989; Gallicano et al., 1993; Anderson et al., 2000).

Term human placenta perfusion studies have demonstrated that placental glucuronidation of zidovudine is nonexistent (Liebes et al., 1990; Schenker et al., 1990; Bawdon et al., 1992) or negligible (Collier et al., 2002). The information on fetal glucuronidation of zidovudine is currently unavailable but is likely to be negligible given the size of the fetal liver (~130 g at GW 40 vs. 1.5kg in adults). Therefore, no irreversible loss of zidovudine was assumed to be present in the fetoplacental unit. Using the estimated zidovudine $CL_{PD,u}$, the predicted MP and UV C-T profiles of zidovudine using the m-f-PBPK model were compared against the observed zidovudine plasma concentrations obtained at labor (O'Sullivan et al., 1993) following a single 200mg oral dose (**Figure 4a**; n=8) or multiple 200mg oral dose preceding an 1-h intravenous infusion (140mg/h) (**Figure 4b**, **c**; n=7). In the latter study, the MP and UV plasma concentrations (observed data) were **simultaneously** obtained from 7 maternal-fetal pairs at the time of birth.

Results

Estimated in vivo transplacental passive diffusion clearance of midazolam

Incorporation of a lag time and optimization of k_a resulted in excellent agreement between the predicted and the observed maternal C-T profiles (**Figure 1a**). Subsequent sensitivity analysis on midazolam $CL_{PD,u}$ demonstrated that though fetal exposure to midazolam was relatively insensitive to changes in $CL_{PD,u}$ (**Supplementary Figure S2**),

a value of 500 L/h [term $f_{u,p}$= 0.045; CL_{PD} of 22.7 L/h) best described the fetal exposure to midazolam (minimum RSS) (**Supplementary Table S1**). The resulting UV plasma concentrations, except for one data point, were in close agreement with the observed UV plasma concentrations, falling within the 90% prediction interval (**Figure 1b**). Additionally, using midazolam as the calibrator, the resultant $CL_{PD,u}$ for theophylline and zidovudine were 342.4 L/h and 216.8 L/h, respectively (**Table 2**).

Theophylline

Using the estimated theophylline $CL_{PD,u}$ of 342.4 L/h (term $f_{u,p}$ = 0.66 ; CL_{PD} = 226.4L/h), the predicted MP (**Figure 2a**) and UV (**Figure 2b**) drug concentrations were in good agreement with the observed data. All predictions (except a single MP concentration) met our verification criterion (i.e. observed plasma concentration fall within the 90% prediction interval). Model prediction precision and bias for MP and UV concentrations were 14.5% and 12.4 % and 9.4% and 7.8%, respectively.

Zidovudine

First, the predicted zidovudine mean plasma C-T profile in non-pregnant population (n=100) was compared against the observed zidovudine mean plasma C-T profiles following various dosing regimens (**Figure 3**). Predicted population mean data matched the observed data with precision of <40% and bias ranging from -30% to 9%. After model verification in non-pregnant population, zidovudine drug-specific parameters were incorporated into our zidovudine m-f-PBPK model. Using the estimated $CL_{PD,u}$ of 216.8 L/h (term $f_{u,p}$=0.8; CL_{PD} = 172.6 L/h, **Table 2**), the predicted MP zidovudine plasma before the onset of labor passed our prediction criterion (**Figure 4a**), whereas the majority of maternal-fetal zidovudine plasma concentrations obtained during delivery fell

within the 90% prediction interval. In the latter scenario, one out of seven MP (**Figure 4b**) and two out of seven UV (**Figure 4c**) plasma concentrations fell outside this 90% prediction interval. Prediction precision and bias for MP and UV plasma drug concentrations were 135.2% and 118.4% and 121.4% and 110.3%, respectively. However, these larger precision and bias data were largely due to the unexpected low MP concentration of 0.17 μg/mL at 43.3h (0.88 h post the initiation of 1-h intravenous infusion to the mother) and consequently low UV concentration. When this point was excluded, the resultant prediction precision and bias for MP and UV drug concentrations reduced considerably to 50.3% and 30.2%, and 28.3 % and 15.0%, respectively.

Discussion

In the current work, we have verified a novel m-f-PBPK model using drugs that passively permeate the placenta and are not known or expected to be metabolized there. Our fetal PBPK model was constructed to be consistent with the distinctive fetal vascular physiology and to allow future incorporation of transport and metabolism within the placenta. The model contains a three compartment placenta consisting of maternal placental blood, placental tissue, and fetal placental blood. The model also accounts for the unique fetal hepatic blood supply as the fetal liver is primarily perfused by umbilical venous blood flow (**Supplementary Figure S1b**). However, a significant portion of the latter (~30-70%) is shunted to the fetal systemic circulation via the ductus venosus. Although this is not the first report of a fetal PBPK model (Yoon et al., 2011; Loccisano et al., 2013; De Sousa Mendes et al., 2016), to our best knowledge it is the first full m-f-PBPK model that: (1) features fetal physiological aspects that are relevant to pharmaceutical drugs, (2) systematically incorporates the gestational age-dependent

changes in maternal drug disposition and maternal-fetal physiology, (3) accounts for the interindividual variability in maternal plasma C-T profile, and (4) well predicts the systemic exposure of test pharmaceutical drugs in maternal-fetal pairs at term.

Crucial for predicting the fetal exposure of passive diffusion drugs is the magnitude of *in vivo* passive diffusion clearance across the placenta. In our model, the mass transfer of a passive diffusion drug from the mother to her fetus is described by equal bidirectional maternal-placental and placental-fetal $CL_{PD,u}$ (Tuntland et al., 1999). In essence, the rate of drug transfer across the placenta is rate-determined by $CL_{PD,u}$ (after accounting for binding) or placental blood flow, whichever is lower. In theory, $CL_{PD,u}$ equals the intrinsic permeability- surface area product. At a given gestational age, the magnitude of this $CL_{PD,u}$ should be directly proportional to its intrinsic permeability (i.e. permeability after adjusting for plasma protein binding) across the syncytiotrophoblast that separates the maternal and fetal circulation. Of note, the differing longitudinal changes in plasma drug binding protein concentrations across the placenta can have a significant impact on the transplacental passage of drugs (Hill and Abramson, 1988; McNamara and Alcorn, 2002) and have been accounted for in our m-f-PBPK model. Despite its importance, IVIVE of passive diffusion clearance across the placenta remains a challenge. Immortalized cell lines of human placenta origin, such as BeWo and Jar, cannot form tight junctions and therefore are poorly suited to estimate placental drug diffusion (Kitano et al., 2004). Although *ex vivo* dually perfused human placentae may represent the most physiologically relevant system, only theophylline and zidovudine have been studied (Liebes et al., 1990; Schenker et al., 1990; Omarini et al., 1992; Dancis et al., 1993). Furthermore, none of these studies provide sufficient data for whole organ scale-up of

$CL_{PD.}$ Therefore, we hypothesized that for a passive diffusion drug, the *in vivo* $CL_{PD,u}$ of the drug can be predicted by calibrating its *in vitro* permeability against the positive control, midazolam. The magnitude of the *in vivo* $CL_{PD,u}$ of a new drug entity can then be calculated assuming that it will be proportional to its passive diffusion permeability relative to that of midazolam in epithelial cell lines that form tight junctions (**Eq.2**).

Our *in vivo* calibrator midazolam [BCS class I (Benet, 2010)] crosses the placenta predominantly via passive diffusion. Due to its high passive membrane permeability, the contribution of P-gp towards midazolam tissue distribution is negligible (Tolle-Sander et al., 2003; Doran et al., 2005). Among the three test compounds, only midazolam maternal population average plasma concentrations (n= 8) have been reported at term. Therefore, midazolam was chosen as our *in vivo* calibrator to estimate the *in vivo* placental diffusion clearance of both theophylline and zidovudine (see next paragraph).

Consistent with our previous findings, a 99% induction in hepatic CYP3A alone was sufficient to explain the clinically observed changes in maternal midazolam disposition during T3 (Ke et al., 2012). As expected, midazolam readily crosses the placenta. The optimized CL_{PD} resulted in all observed UV midazolam plasma concentrations falling within the 90% prediction interval, suggesting ~50% extraction ratio by the placenta and a blood-flow limited extraction by the fetal placental flow (CL_{PD} of 22.7 L/h vs. term placental and umbilical venous blood flows of ~50L/h and ~20L/h, respectively)(Abduljalil et al., 2012). The first verification drug, theophylline, is also a BCS class I drug (Benet, 2010). So far, only OAT2 has been indicated in theophylline tissue uptake but this transporter is absent in human placenta (Kobayashi et al., 2005; Mao et al., 2014). Theophylline is mainly cleared by CYP1A2 with minor contributions

from CYP 3A and CYP2E1 as well as a small renal component (Ke et al., 2013a). The incorporation of a 65% reduction in maternal hepatic CYP1A2 activity (Tracy et al., 2005) along with the 99% increase in CYP3A activity satisfactorily explained the observed maternal plasma concentrations (**Figure 2a**). Using the predicted $CL_{PD,u}$ of 342.4 L/h , fetal exposure was well described (**Figure 2b**). Of note, as a result of moderate protein binding ($f_{u,p}$= 0.66 at term) and relatively high $CL_{PD,u}$ (342.4L/h), the predicted transplacental passage of theophylline was blood-flow limited.

The second verification drug, zidovudine, is a nucleoside reverse transcriptase inhibitor and structure analogue of thymidine. Although it has been shown to be transported *in vitro* by several transporters expressed in human placenta (e.g. P-gp, BCRP, MRP5, ENT2, and OAT4), several *ex vivo* placenta perfusion studies indicate that zidovudine crosses the placental via passive diffusion (Liebes et al., 1990; Schenker et al., 1990; Dancis et al., 1993). Zidovudine is mainly cleared by UGT2B7 *in vivo* with a renal clearance exceeding renal filtration. The activity of UGT2B7 is generally regarded not to be affected by pregnancy (Anderson, 2005) and is supported by our simulation. Several studies have shown that zidovudine is a substrate for human OATs (i.e.OAT1-4), all of which are expressed in the kidney (Takeda et al., 2002). Interestingly, although pregnancy may increase renal OAT1 activity [measured by the 55% increase in amoxicillin net renal secretion during T3, which may be attributed to enhanced renal OAT1 activity and/or reduced reabsorption(Andrew et al., 2007)], our simulations demonstrated that maternal physiological changes along with the increased renal filtration clearance sufficiently described the maternal disposition of zidovudine (**Figure 4a** and **b**).

zidovudine demonstrates good permeability and was also estimated to have blood flow limited distribution into the fetal compartment.

Overall, the m-f-PBPK model described well-predicted the maternal and fetal exposure to the two verification drugs (zidovudine and theophylline) at term across a range of dosing regimens, with nearly all simulated plasma drug concentrations falling within the 90% prediction interval in both the mother and her fetus. Because these drugs passively diffuse across the placenta and are not significantly metabolized in the fetal liver or the placenta, the overall unbound fetal plasma AUC is predicted to be equal to the unbound maternal plasma AUC. Due to the sparse fetal plasma drug concentration data available, the fetal AUC of the verification drugs could not be estimated. Therefore our goal was to dynamically predict the fetal plasma drug concentrations available in the literature (i.e. predict the time-variant fetal plasma C-T curve). Such dynamic prediction is a true test of any m-f-PBPK model, including when the drugs are extensively transported or metabolized in the placenta. In the future, our aim is to incorporate these processes in our model when quantification data on expression of placental transporters and enzymes are available.

The application of PBPK models for predicting drug disposition in the coupled maternal-fetal pairs is still in its infancy. Therefore, as is the case with other PBPK models, our model has several limitations. First, while interindividual variability in maternal drug disposition, where available, was incorporated in our model, due to lack of data on variability of feto-placental parameters, such variability could not be incorporated in the model. This may underestimate the true variability in fetal exposure. Second, midazolam was not a sensitive calibrator and both theophylline and zidovudine

demonstrated blood flow limited passive placental diffusion clearance. Ideally, the passive diffusion clearance of test compounds should span a much wider range, including hydrophilic drugs that passively diffuse across the placenta with CL_{PD} much lower than the placental blood flow. Unfortunately, coupled maternal-fetal PK data for such drugs are not available. Third, until placental transporters and metabolic enzyme expression are available our model can be applied to only drugs that passively diffuse across the placenta and are not metabolized/transported there. To address this limitation, proteomics based quantification of placental transporters and enzymes is currently underway in our laboratory. Fourth, although our model can predict fetal exposure to passive diffusion drugs across fetal developmental stages (GW 14-term), many fetal physiological parameters are not available for the first half of pregnancy, and the fetal skin is not keratinized at < 20 weeks of gestation (Polin et al., 2004), potentially enabling the bidirectional diffusion of drugs through the fetal skin to the amniotic fluid. While the latter limitation can be overcome by including such a possibility in a future version of our model, the former issue reflects an inherent limitation of studies in this special population. That is, the difficulty of measuring physiological parameters of the fetus at earlier gestational age. As a result, we have less confidence in predicting fetal drug exposure at < 20 weeks of gestation.

The clinical implications of the current study relate to addressing an urgent need for an understudied and vulnerable population: quantitative assessment of fetal exposure to drugs in the maternal-fetal dyad. Drug use during pregnancy is a reality. When the perceived benefits outweigh risks, pharmacotherapy of pregnant women is initiated, in most cases, without prior knowledge on the maternal-fetal disposition of the drug.

However, obtaining any fetal drug exposure data at term is fraught with logistical and ethical issues. As a result, there is a paucity of fetal exposure data in this population. As pointed out earlier, existing fetal drug exposure data are limited to term pregnancy. Furthermore, such data for early gestational age fetuses are virtually impossible to obtain, rendering fetuses orphan population with respect to drug exposure and drug efficacy/toxicity. Instead, the proposed PBPK model can provide information on fetal drug exposure based on sound physiological data and modeling. While data to verify our model for gestational ages other than term are not available, the term verification data presented above lends considerable confidence that our m-f-PBPK model can be used to *a priori* predict fetal exposure to drugs (that passively diffuse across the placenta) during pregnancy. When earlier gestational age data become available, our model can be verified for these gestational ages.

Acknowledgements

The authors would like to thank Drs. Nina Isoherranen and Kazuya Ishida for their insightful comments.

Authorship Contributions

Participated in research design: Zhang and Unadkat

Conducted experiments: Zhang

Contributed new reagents or analytic tools: NA

Performed data analysis: Zhang

Wrote or contributed to the writing of the manuscript: Zhang and Unadkat

Conflict of Interest/Disclosure

The authors declared no conflict of interest.

References

Abduljalil K, Furness P, Johnson TN, Rostami-Hodjegan A, and Soltani H (2012) Anatomical, physiological and metabolic changes with gestational age during normal pregnancy: a database for parameters required in physiologically based pharmacokinetic modelling. *Clin Pharmacokinet* **51:**365-396.

Andersin R (1991) Solubility and acid-base behaviour of midazolam in media of different pH, studied by ultraviolet spectrophotometry with multicomponent software. *J Pharm Biomed Anal* **9:**451-455.

Anderson GD (2005) Pregnancy-induced changes in pharmacokinetics: a mechanistic-based approach. *Clin Pharmacokinet* **44:**989-1008.

Anderson PL, Noormohamed SE, Henry K, Brundage RC, Balfour HH, and Fletcher CV (2000) Semen and serum pharmacokinetics of zidovudine and zidovudine-glucuronide in men with HIV-1 infection. *Pharmacotherapy* **20:**917-922.

Andrew MA, Easterling TR, Carr DB, Shen D, Buchanan ML, Rutherford T, Bennett R, Vicini P, and Hebert MF (2007) Amoxicillin pharmacokinetics in pregnant women: modeling and simulations of dosage strategies. *Clin Pharmacol Ther* **81:**547-556.

Aslaksen A, Bakke OM, and Vigander T (1981) Comparative pharmacokinetics of theophylline and aminophylline in man. *Br J Clin Pharmacol* **11:**269-273.

Barter ZE, Bayliss MK, Beaune PH, Boobis AR, Carlile DJ, Edwards RJ, Houston JB, Lake BG, Lipscomb JC, Pelkonen OR, Tucker GT, and Rostami-Hodjegan A (2007) Scaling factors for the extrapolation of in vivo metabolic drug clearance

from in vitro data: reaching a consensus on values of human microsomal protein and hepatocellularity per gram of liver. *Curr Drug Metab* **8:**33-45.

Barter ZE, Chowdry JE, Harlow JR, Snawder JE, Lipscomb JC, and Rostami-Hodjegan A (2008) Covariation of human microsomal protein per gram of liver with age: absence of influence of operator and sample storage may justify interlaboratory data pooling. *Drug Metab Dispos* **36:**2405-2409.

Bawdon RE, Sobhi S, and Dax J (1992) The transfer of anti-human immunodeficiency virus nucleoside compounds by the term human placenta. *Am J Obstet Gynecol* **167:**1570-1574.

Benet LZ (2010) Predicting Drug Disposition via Application of a Biopharmaceutics Drug Disposition Classification System. *Basic Clin Pharmacol Toxicol* **106:**162-167.

Chung W (2004), in: *Teratogens and Their Effects*, http://www.columbia.edu/itc/hs/medical/humandev/2004/Chpt23-Teratogens.pdf.

Cload PA (1989) A review of the pharmacokinetics of zidovudine in man. *J Infect* **18 Suppl 1:**15-21.

Collett A, Stephens RH, Harwood MD, Humphrey M, Dallman L, Bennett J, Davis J, Carlson GL, and Warhurst G (2008) Investigation of regional mechanisms responsible for poor oral absorption in humans of a modified release preparation of the alpha-adrenoreceptor antagonist, 4-amino-6,7-dimethoxy-2-(5-methanesulfonamido-1,2,3,4 tetrahydroisoquinol-2-yl)-5-(2-pyridyl)quinazoline (UK-338,003): the rational use of ex vivo intestine to predict in vivo absorption. *Drug Metab Dispos* **36:**87-94.

Collier AC, Ganley NA, Tingle MD, Blumenstein M, Marvin KW, Paxton JW, Mitchell MD, and Keelan JA (2002) UDP-glucuronosyltransferase activity, expression and cellular localization in human placenta at term. *Biochem Pharmacol* **63:**409-419.

Collins JM and Unadkat JD (1989) Clinical pharmacokinetics of zidovudine. An overview of current data. *Clin Pharmacokinet* **17:**1-9.

Cretton EM, Xie MY, Bevan RJ, Goudgaon NM, Schinazi RF, and Sommadossi JP (1991) Catabolism of 3'-azido-3'-deoxythymidine in hepatocytes and liver microsomes, with evidence of formation of 3'-amino-3'-deoxythymidine, a highly toxic catabolite for human bone marrow cells. *Mol Pharmacol* **39:**258-266.

Dancis J, Lee J, Mendoza S, and Liebes L (1993) Nucleoside transport by perfused human placenta. *Placenta* **14:**547-554.

De Sousa Mendes M, Hirt D, Vinot C, Valade E, Lui G, Pressiat C, Bouazza N, Foissac F, Blanche S, Le MP, Peytavin G, Treluyer JM, Urien S, and Benaboud S (2016) Prediction of human fetal pharmacokinetics using ex vivo human placenta perfusion studies and physiologically based models. *Br J Clin Pharmacol* **81:**646-657.

de Souza J, Benet LZ, Huang Y, and Storpirtis S (2009) Comparison of bidirectional lamivudine and zidovudine transport using MDCK, MDCK-MDR1, and Caco-2 cell monolayers. *J Pharm Sci* **98:**4413-4419.

Di L, Whitney-Pickett C, Umland JP, Zhang H, Zhang X, Gebhard DF, Lai Y, Federico JJ, Davidson RE, Smith R, Reyner EL, Lee C, Feng B, Rotter C, Varma MV, Kempshall S, Fenner K, El-Kattan AF, Liston TE, and Troutman MD (2011)

Development of a new permeability assay using low-efflux MDCKII cells. *J Pharm Sci* **100:**4974-4985.

Doran A, Obach RS, Smith BJ, Hosea NA, Becker S, Callegari E, Chen C, Chen X, Choo E, Cianfrogna J, Cox LM, Gibbs JP, Gibbs MA, Hatch H, Hop CE, Kasman IN, Laperle J, Liu J, Liu X, Logman M, Maclin D, Nedza FM, Nelson F, Olson E, Rahematpura S, Raunig D, Rogers S, Schmidt K, Spracklin DK, Szewc M, Troutman M, Tseng E, Tu M, Van Deusen JW, Venkatakrishnan K, Walens G, Wang EQ, Wong D, Yasgar AS, and Zhang C (2005) The impact of P-glycoprotein on the disposition of drugs targeted for indications of the central nervous system: evaluation using the MDR1A/1B knockout mouse model. *Drug Metab Dispos* **33:**165-174.

Evans MI, Pryde PG, Reichler A, Bardicef M, and Johnson MP (1993) Fetal drug therapy. *Western Journal of Medicine* **159:**325-332.

Furukawa T, Naritomi Y, Tetsuka K, Nakamori F, Moriguchi H, Yamano K, Terashita S, Tabata K, and Teramura T (2014) Species differences in intestinal glucuronidation activities between humans, rats, dogs and monkeys. *Xenobiotica* **44:**205-216.

Gallicano K (2000) Antiretroviral-Drug Concentrations in Semen. *Antimicrob Agents Chemother* **44:**1117-1118.

Gallicano K, Sahai J, Ormsby E, Cameron DW, Pakuts A, and McGilveray I (1993) Pharmacokinetics of zidovudine after the initial single dose and during chronic-dose therapy in HIV-infected patients. *Br J Clin Pharmacol* **36:**128-131.

Gaohua L, Abduljalil K, Jamei M, Johnson TN, and Rostami-Hodjegan A (2012) A pregnancy physiologically based pharmacokinetic (p-PBPK) model for disposition of drugs metabolized by CYP1A2, CYP2D6 and CYP3A4. *Br J Clin Pharmacol* **74:**873-885.

Gertz M, Harrison A, Houston JB, and Galetin A (2010) Prediction of human intestinal first-pass metabolism of 25 CYP3A substrates from in vitro clearance and permeability data. *Drug Metab Dispos* **38:**1147-1158.

Ha HR, Chen J, Freiburghaus AU, and Follath F (1995) Metabolism of theophylline by cDNA-expressed human cytochromes P-450. *Br J Clin Pharmacol* **39:**321-326.

Hardman HF (1962) Molecular form of theophylline responsible for positive inotropic activity. *Circ Res* **10:**598-607.

Hebert MF, Easterling TR, Kirby B, Carr DB, Buchanan ML, Rutherford T, Thummel KE, Fishbein DP, and Unadkat JD (2008) Effects of pregnancy on CYP3A and P-glycoprotein activities as measured by disposition of midazolam and digoxin: a University of Washington specialized center of research study. *Clin Pharmacol Ther* **84:**248-253.

Hill MD and Abramson FP (1988) The significance of plasma protein binding on the fetal/maternal distribution of drugs at steady-state. *Clin Pharmacokinet* **14:**156-170.

Howe JL, Back DJ, and Colbert J (1992) Extrahepatic metabolism of zidovudine. *Br J Clin Pharmacol* **33:**190-192.

Irvine JD, Takahashi L, Lockhart K, Cheong J, Tolan JW, Selick HE, and Grove JR (1999) MDCK (Madin-Darby canine kidney) cells: A tool for membrane permeability screening. *J Pharm Sci* **88:**28-33.

Kanto J, Sjovall S, Erkkola R, Himberg JJ, and Kangas L (1983) Placental transfer and maternal midazolam kinetics. *Clin Pharmacol Ther* **33:**786-791.

Ke AB, Nallani SC, Zhao P, Rostami-Hodjegan A, Isoherranen N, and Unadkat JD (2013a) A physiologically based pharmacokinetic model to predict disposition of CYP2D6 and CYP1A2 metabolized drugs in pregnant women. *Drug Metab Dispos* **41:**801-813.

Ke AB, Nallani SC, Zhao P, Rostami-Hodjegan A, and Unadkat JD (2012) A PBPK Model to Predict Disposition of CYP3A-Metabolized Drugs in Pregnant Women: Verification and Discerning the Site of CYP3A Induction. *CPT Pharmacometrics Syst Pharmacol* **1:**e3.

Ke AB, Nallani SC, Zhao P, Rostami-Hodjegan A, and Unadkat JD (2013b) Expansion of a PBPK Model to Predict Disposition in Pregnant Women of Drugs Cleared via Multiple CYP Enzymes, Including CYP2B6, CYP2C9 and CYP2C19. *Br J Clin Pharmacol*.

Kilford PJ, Stringer R, Sohal B, Houston JB, and Galetin A (2009) Prediction of drug clearance by glucuronidation from in vitro data: use of combined cytochrome P450 and UDP-glucuronosyltransferase cofactors in alamethicin-activated human liver microsomes. *Drug Metab Dispos* **37:**82-89.

Kitano T, Iizasa H, Hwang I-W, Hirose Y, Morita T, Maeda T, and Nakashima E (2004) Conditionally immortalized syncytiotrophoblast cell lines as new tools for study of the blood-placenta barrier. *Biol Pharm Bull* **27:**753-759.

Klecker RW, Jr., Collins JM, Yarchoan R, Thomas R, Jenkins JF, Broder S, and Myers CE (1987) Plasma and cerebrospinal fluid pharmacokinetics of 3'-azido-3'-deoxythymidine: a novel pyrimidine analog with potential application for the treatment of patients with AIDS and related diseases. *Clin Pharmacol Ther* **41:**407-412.

Knights KM, Spencer SM, Fallon JK, Chau N, Smith PC, and Miners JO (2016) Scaling factors for the in vitro-in vivo extrapolation (IV-IVE) of renal drug and xenobiotic glucuronidation clearance. *Br J Clin Pharmacol*.

Kobayashi Y, Sakai R, Ohshiro N, Ohbayashi M, Kohyama N, and Yamamoto T (2005) Possible involvement of organic anion transporter 2 on the interaction of theophylline with erythromycin in the human liver. *Drug Metab Dispos* **33:**619-622.

Liebes L, Mendoza S, Wilson D, and Dancis J (1990) Transfer of zidovudine (AZT) by human placenta. *J Infect Dis* **161:**203-207.

Loccisano AE, Longnecker MP, Campbell JL, Andersen ME, and Clewell HJ (2013) Development of PBPK models for PFOA and PFOS for human pregnancy and lactation life stages. *J Toxicol Environ Health A* **76:**25-57.

Mahar Doan KM, Humphreys JE, Webster LO, Wring SA, Shampine LJ, Serabjit-Singh CJ, Adkison KK, and Polli JW (2002) Passive permeability and P-glycoprotein-

mediated efflux differentiate central nervous system (CNS) and non-CNS marketed drugs. *J Pharmacol Exp Ther* **303:**1029-1037.

Mao Q, Ganapathy V, and Unadkat JD (2014) Drug Transport in the Placenta, in: *Drug Transporters*, pp 341-353, John Wiley & Sons, Inc.

Masungi C, Borremans C, Willems B, Mensch J, Van Dijck A, Augustijns P, Brewster ME, and Noppe M (2004) Usefulness of a novel Caco-2 cell perfusion system. I. In vitro prediction of the absorption potential of passively diffused compounds. *J Pharm Sci* **93:**2507-2521.

McNamara PJ and Alcorn J (2002) Protein binding predictions in infants. *AAPS PharmSci* **4:**E4.

Mitchell AA, Gilboa SM, Werler MM, Kelley KE, Louik C, and Hernandez-Diaz S (2011) Medication use during pregnancy, with particular focus on prescription drugs: 1976-2008. *Am J Obstet Gynecol* **205:**51 e51-58.

O'Sullivan MJ, Boyer PJ, Scott GB, Parks WP, Weller S, Blum MR, Balsley J, and Bryson YJ (1993) The pharmacokinetics and safety of zidovudine in the third trimester of pregnancy for women infected with human immunodeficiency virus and their infants: phase I acquired immunodeficiency syndrome clinical trials group study (protocol 082). Zidovudine Collaborative Working Group. *Am J Obstet Gynecol* **168:**1510-1516.

Ohno S and Nakajin S (2009) Determination of mRNA expression of human UDP-glucuronosyltransferases and application for localization in various human tissues by real-time reverse transcriptase-polymerase chain reaction. *Drug Metab Dispos* **37:**32-40.

Omarini D, Barzago MM, Aramayona J, Bortolotti A, Lucchini G, and Bonati M (1992) Theophylline transfer across human placental cotyledon during in vitro dual perfusion. *J Med* **23:**101-116.

Polin RA, Fox WW, and Abman SH (2004) *Fetal and neonatal physiology*. W.B. Saunders Co., Philadelphia, Pa.

Ron M, Hochner-Celnikier D, Menczel J, Palti Z, and Kidroni G (1984) Maternal-fetal transfer of aminophylline. *Acta Obstet Gynecol Scand* **63:**217-218.

Schenker S, Johnson RF, King TS, Schenken RS, and Henderson GI (1990) Azidothymidine (zidovudine) transport by the human placenta. *The American journal of the medical sciences* **299:**16-20.

Sheiner LB and Beal SL (1985) Pharmacokinetic parameter estimates from several least squares procedures: superiority of extended least squares. *J Pharmacokinet Biopharm* **13:**185-201.

Shuster DL, Risler LJ, Prasad B, Calamia JC, Voellinger JL, Kelly EJ, Unadkat JD, Hebert MF, Shen DD, Thummel KE, and Mao Q (2014) Identification of CYP3A7 for glyburide metabolism in human fetal livers. *Biochem Pharmacol* **92:**690-700.

Stagg MP, Cretton EM, Kidd L, Diasio RB, and Sommadossi JP (1992) Clinical pharmacokinetics of 3'-azido-3'-deoxythymidine (zidovudine) and catabolites with formation of a toxic catabolite, 3'-amino-3'-deoxythymidine. *Clin Pharmacol Ther* **51:**668-676.

Takeda M, Khamdang S, Narikawa S, Kimura H, Kobayashi Y, Yamamoto T, Cha SH, Sekine T, and Endou H (2002) Human organic anion transporters and human

organic cation transporters mediate renal antiviral transport. *J Pharmacol Exp Ther* **300:**918-924.

Tasnif Y, Morado J, and Hebert MF (2016) Pregnancy-Related Pharmacokinetic Changes. *Clin Pharmacol Ther*.

Taub ME, Kristensen L, and Frokjaer S (2002) Optimized conditions for MDCK permeability and turbidimetric solubility studies using compounds representative of BCS classes I-IV. *Eur J Pharm Sci* **15:**331-340.

Teksin ZS, Seo PR, and Polli JE (2010) Comparison of drug permeabilities and BCS classification: three lipid-component PAMPA system method versus Caco-2 monolayers. *AAPS J* **12:**238-241.

Tolle-Sander S, Rautio J, Wring S, Polli JW, and Polli JE (2003) Midazolam exhibits characteristics of a highly permeable P-glycoprotein substrate. *Pharm Res* **20:**757-764.

Tracy TS, Venkataramanan R, Glover DD, and Caritis SN (2005) Temporal changes in drug metabolism (CYP1A2, CYP2D6 and CYP3A Activity) during pregnancy. *Am J Obstet Gynecol* **192:**633-639.

Tuntland T, Odinecs A, Pereira CM, Nosbisch C, and Unadkat JD (1999) In vitro models to predict the in vivo mechanism, rate, and extent of placental transfer of dideoxynucleoside drugs against human immunodeficiency virus. *Am J Obstet Gynecol* **180:**198-206.

Veal GJ, Wild MJ, Barry MG, and Back DJ (1994) Effects of dideoxyinosine and dideoxycytidine on the intracellular phosphorylation of zidovudine in human mononuclear cells. *Br J Clin Pharmacol* **38:**323-328.

Williams JA, Ring BJ, Cantrell VE, Jones DR, Eckstein J, Ruterbories K, Hamman MA, Hall SD, and Wrighton SA (2002) Comparative metabolic capabilities of CYP3A4, CYP3A5, and CYP3A7. *Drug Metab Dispos* **30:**883-891.

Yamashita S, Furubayashi T, Kataoka M, Sakane T, Sezaki H, and Tokuda H (2000) Optimized conditions for prediction of intestinal drug permeability using Caco-2 cells. *Eur J Pharm Sci* **10:**195-204.

Yazdanian M, Glynn SL, Wright JL, and Hawi A (1998) Correlating partitioning and caco-2 cell permeability of structurally diverse small molecular weight compounds. *Pharm Res* **15:**1490-1494.

Yoon M, Schroeter JD, Nong A, Taylor MD, Dorman DC, Andersen ME, and Clewell HJ, 3rd (2011) Physiologically based pharmacokinetic modeling of fetal and neonatal manganese exposure in humans: describing manganese homeostasis during development. *Toxicol Sci* **122:**297-316.

Zhang H, Wu X, Wang H, Mikheev AM, Mao Q, and Unadkat JD (2008) Effect of pregnancy on cytochrome P450 3a and P-glycoprotein expression and activity in the mouse: mechanisms, tissue specificity, and time course. *Mol Pharmacol* **74:**714-723.

Footnote:

The current work is funded by National Institutes of Health National Institute on Drug Abuse [Grant P01DA032507].

Figure Legends

Figure 1: Predicted population mean (black solid lines) and observed (open circles) maternal plasma (MP) (**a**) and umbilical venous (UV) plasma (**b**) midazolam concentration-time (C-T) profiles following a single 15 mg oral dose at term (gestational week 40) (Kanto et al., 1983). In **a**, the predicted MP C-T profile is overlaid with the observed maternal plasma data (mean ± SD; n=8). In **b**, each observed UV data point was derived from a single maternal-fetal pair from another subset of subjects from the same study (n=7). The observed mean midazolam MP concentrations at various time points fell within the 90% prediction interval (5^{th}-95^{th} percentile boundaries; lower and upper grey dashed lines, respectively) for a virtual population consisting of 100 maternal-fetal pairs (**a**). Consequently, all the observed individual UV plasma concentrations were within the 90% prediction interval (**b**). Insets are the same data plotted to highlight early time points.

Figure 2: Predicted population mean (black solid lines) and observed (open circles) maternal plasma (MP) (**a**) and umbilical venous (UV) plasma (**b**) theophylline concentration-time (C-T) profiles following 5 doses of 160mg oral theophylline every 6 hour at term (gestational week 40) (Ron et al, 1984). Each pair of observed data (MP and UV) were derived from a single maternal-fetal pair (n=10). All observed MP drug concentrations, except one data point, and subsequently all observed UV data points, fell within the 90% prediction interval (5^{th}-95^{th} percentile boundaries ; lower and upper grey dashed lines, respectively), for a virtual population consisting of 100 maternal-fetal pairs.

Figure 3: Predicted population mean (black sloid lines) and observed (open circles) plasma zidovudine concentration-time (C-T) profiles following a 1-h 2.5mg/kg

intravenous infusion dose (**a**; Clod et al., 1989, n=8) or a single 200mg oral dose (**b**; Gallicano et al., 1993, n=10) or a single 300mg oral dose (**c**; Anderson et al., 2000, n=4) in non-pregnant population. All observed plasma drug concentrations (mean ± SD), except one terminal phase plasma concentration (**a**), in HIV-infected asymptomatic male volunteers fell within the 90% prediction interval (5^{th}-95^{th} percentile boundaries; lower and upper grey dashed lines, respectively) from a virtual population consisting of 100 male subjects.

Figure 4: Predicted population mean (black solid lines) and observed (open circles) maternal plasma (MP) (**a** and **b**) and umbilical venous (UV) plasma (**c**) zidovudine concentration-time (C-T) profiles in HIV-infected, asymptomatic women with uncomplicated pregnancies (O'Sullivan et al., 1993). 200 mg oral dose of zidovudine (5 times per day) was commenced prior to onset of labor (**a**; gestational week 40, n=8) . Then, during labor, 4 hours after last dose, 140mg zidovudine was intravenously infused over 1-h period to the same volunteers (**b** and **c**; n=7). In **a**, all the observed mean maternal plasma zidovudine concentrations fell within the 90% prediction interval (5^{th} and 95^{th} percentile range; lower and upper grey dashed lines, respectively) for a virtual population consisting of 100 maternal-fetal pairs. In **b** and **c**, each data point (open circles) represents an observed single time point zidovudine concentration from paired MP (**b**) or UV plasma (**c**) samples. Six out of seven observed MP concentrations, and five out of seven UV plasma concentrations, fell within the 90% prediction interval. Insets are the same data replotted with a different time scale to illustrate the dosing regimen.

Table 1: Summary of midazolam, theophylline, and zidovudine drug-dependent parameters

Parameter	Midazolam value	Methods/ reference	Theophylline value	Methods/ reference	Zidovudine value	Methods/ reference
Molecular Weight	325.8	Library[a]	180.2	Library[a]	267.2	Library[a]
Log $P_{o:w}$	3.13	Optimized[b]	-0.02	Library[a]	0.05	Library[a]
pKa	10.95,6.2	Library[a]	8.8,0.99	Library[a]	9.70	Library[a]
B/P Ratio	0.66	Library[a]	0.82	Library[a]	0.91	Library[a]
$f_{u,p}$*	0.032	Library[a]	0.58	Library[a]	0.80	Library[a]
F_a	0.88	Library[a]	0.97	Library[a]	0.83	Predicted by ADAM model
k_a (h^{-1})	4.0	Optimized[c]	1.0	Reported [e]	4.05	Reported[f]
F_g	0.58	Library[a]	1.0	Reported[d]	1	Assumed[g]
V_{ss} (L/Kg)	1.1	Reported[d]	0.39	Reported[d]	1.10	Optimized[h]
CL_{iv}(L/h)	23.0	Library[a]	3.0	Reported[d]	91.0	Reported[i]
CL_r(L/h)	0.085	Library[a]	0.45	Reported[d]	15.5	Library
$CL_{hep,int,u}$(L/h)	1672.3	Library[a]	4.60	Reported[d]	30.9	Calculated[k]
f_m and f_e	$f_{m,3A}$ =92%, $f_e \approx 0\%$	Reported[d]	$f_{m,1A2}$ =68%, $f_{m,3A}$ =7%, $f_{m,2E1}$ =10%, f_e =15%	Reported[d]	$f_{m,UGT2B7}$=67%, f_e =17%	Reported[l]

*: Note $f_{u,p}$ refers to the reported unbound fraction of drug in plasma in non-pregnant population.

**: Midazolam is a weak base (Andersin, 1991), whereas zidovudine is a weak acid (Gallicano, 2000). In contrast, theophylline is neutral (Hardman, 1962).

[a]: Refers to the SimCYP Simulator® compound library (version 14).

[b]: Previously optimized and validated to match the predicted V_{ss} to the reported V_{ss} value of 1.10 L/kg in the literature (Ke et al., 2012).

[c]: Optimized based on sensitivity analysis to match reported absorption in pregnant subjects(Kanto et al., 1983) .

[d]: Validated literature value used in our pregnancy PBPK model (Ke et al., 2012; Ke et al., 2013a).

[e]: Phoenix® estimate from reported oral absorption data in healthy volunteers (Aslaksen et al., 1981).

[f]: Phoenix® estimate from reported oral absorption data in non-pregnant subjects (Klecker et al., 1987).

[g]: No report on zidovudine F_g is available. zidovudine F_g was assumed 1 since human intestinal microsomes showed negligible UGT2B7 activity measured by two UGT2B7 probes (diclofenac and gemfibrozil) (Furukawa et al., 2014).

[h]: The reported average zidovudine V_{ss} is 1.4 ± 0.4 L/kg.(Collins and Unadkat, 1989) This V_{ss} was optimized through manual sensitivity analysis in the range of 0.8-1.6 L/kg to match the predicted peak plasma concentration (C_{max}) to the reported C_{max} following intravenous infusion in non-pregnant population (Klecker et al., 1987; Cload, 1989).

[i]: Calculated based on the reported average intravenous clearance of 1.3 L/h/kg assuming 70kg body weight.

[j]: Reported zidovudine fraction excreted in the urine (f_e) ranges from 14% to 20%. The SimCYP Simulator® compound library value of 15.5L/h (f_e =17%) was used.

[k]: Back calculation from well-stirred liver model using hepatic blood flow of 90 L/h assuming hepatic clearance of 20.7 L/h.

[l]: Estimated from urinary data (Cload, 1989; Stagg et al., 1992).

Table 2: P_{app} values from *in vitro* studies and the estimated $CL_{PD,u}$'s

Drug	**P_{app} value ($nm \cdot s^{-1}$)**				**$CL_{PD,u}$ (L/h)**	**Resources**
	Mean	S.D	Median	Range		
Midazolam	489.9	158.7	490.0	320 - 699	500.0	(Yamashita et al., 2000; Mahar Doan et al., 2002; Taub et al., 2002; Tolle-Sander et al., 2003; Gertz et al., 2010)
Theophylline	335.5	162.2	260.0	231-620	342.4	(Yamashita et al., 2000; Masungi et al., 2004; Collett et al., 2008; Teksin et al., 2010; Di et al., 2011)
Zidovudine	212.4	217.2	69.3	31.8-543.3	216.8	(Yazdanian et al., 1998; Irvine et al., 1999; de Souza et al., 2009)

a. The predicted theophylline maternal fu,p is 0.66 at term; b. The predicted zidovudine maternal fu,p is ~0.80 at term; c. The estimated CLPD,u values were based on mean Papp from *in vitro* reports.

Figure 1

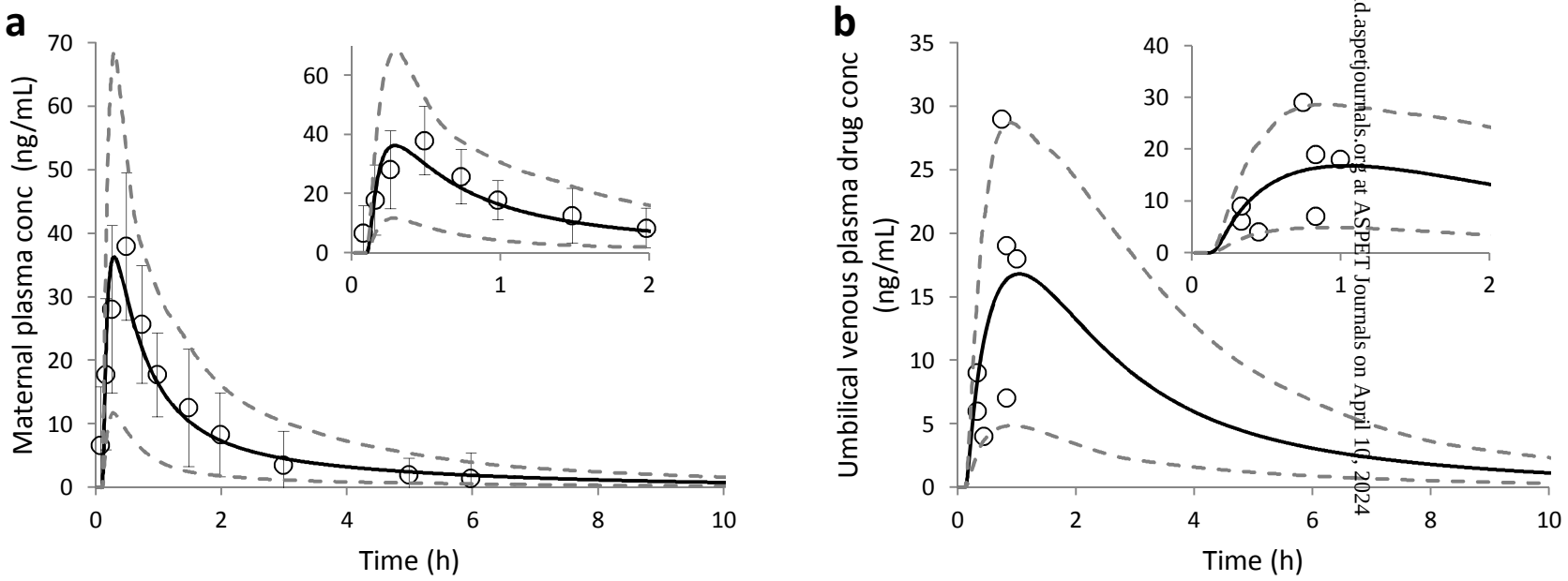


Figure 2

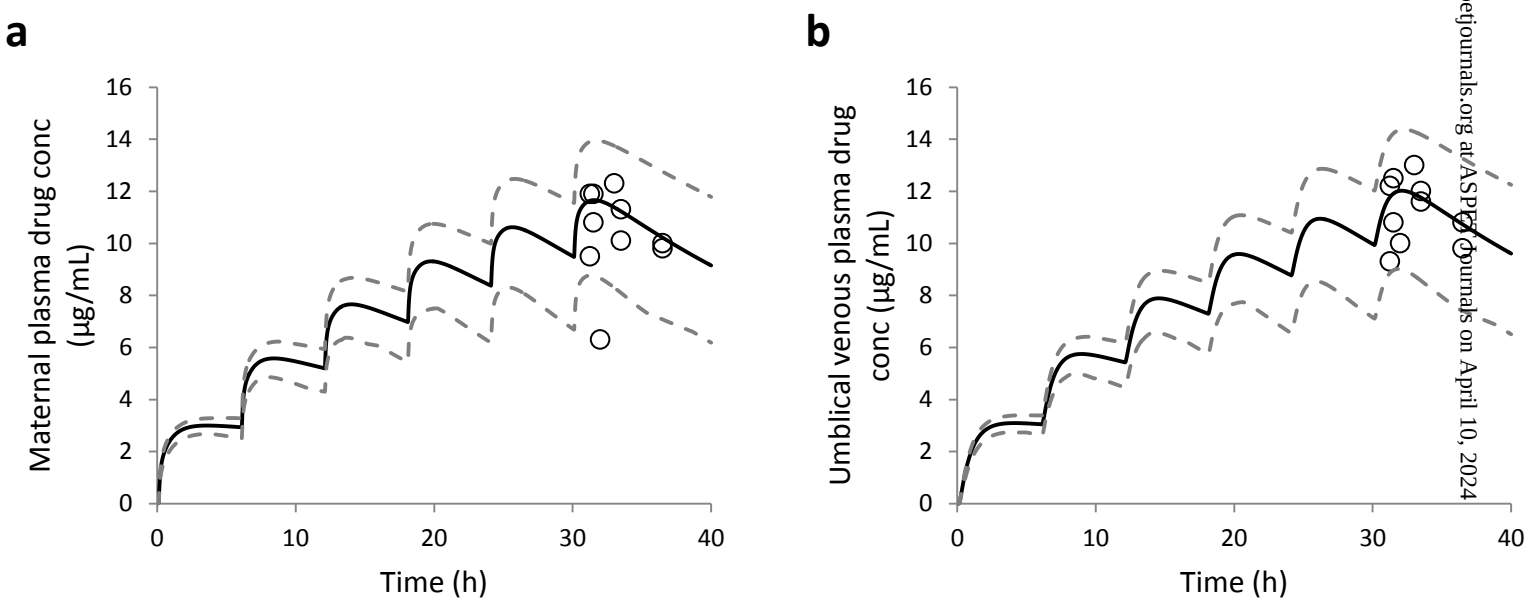


Figure 3

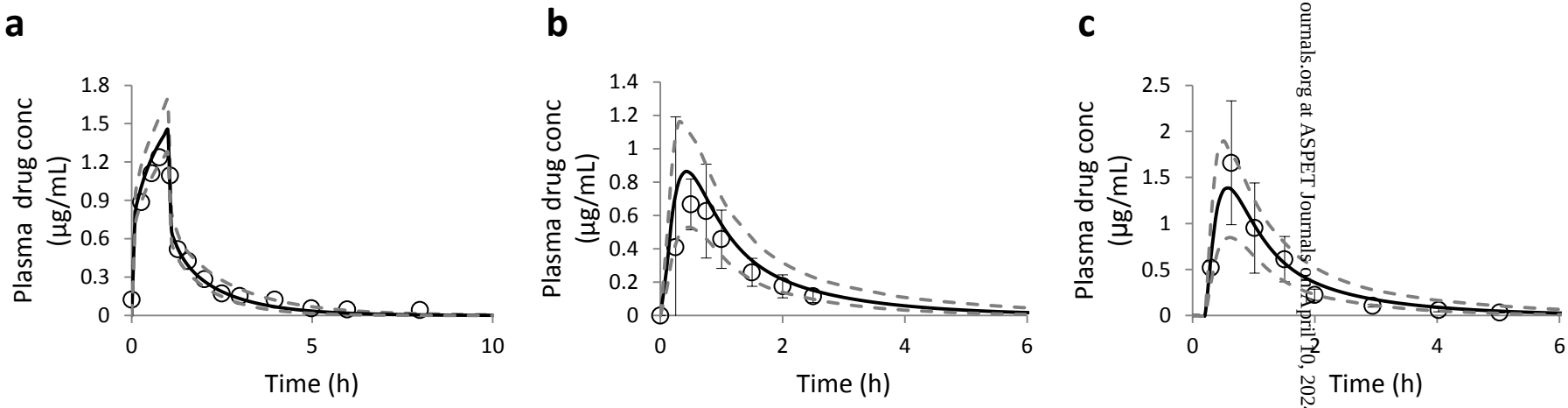
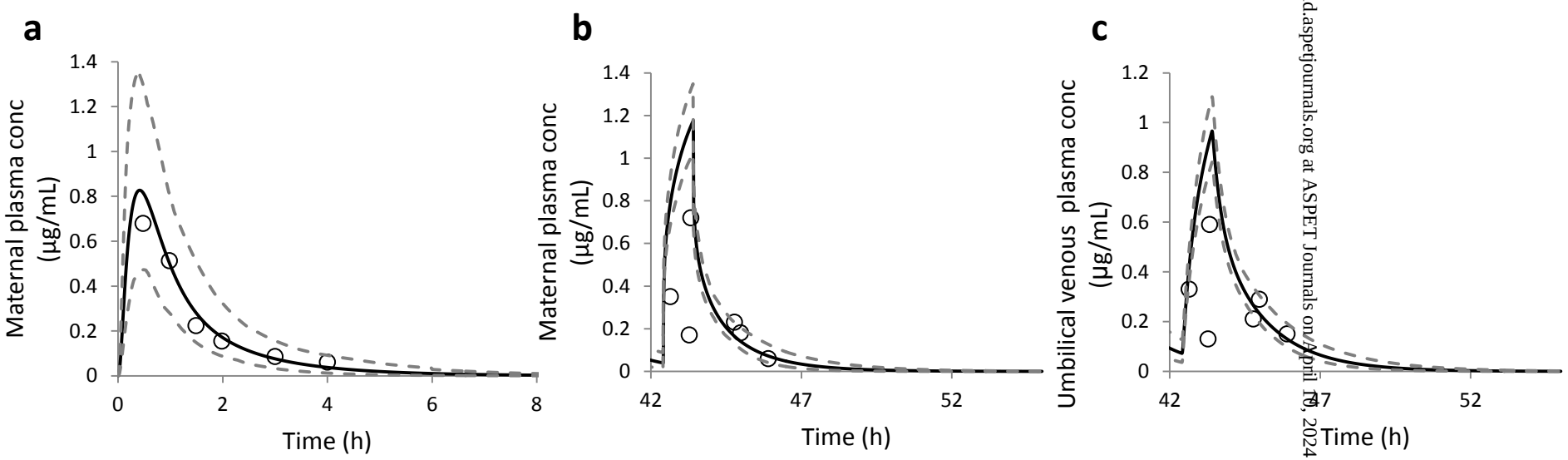


Figure 4



DMD#73957

Drug Metabolism and Disposition

Supplementary Information

Verification of a Maternal-Fetal Physiologically Based Pharmacokinetic Model for Passive Placental Permeability Drugs

Zufei Zhang and Jashvant D. Unadkat

Department of Pharmaceutics, University of Washington, Seattle, WA

Supplementary Figures

Figure S1

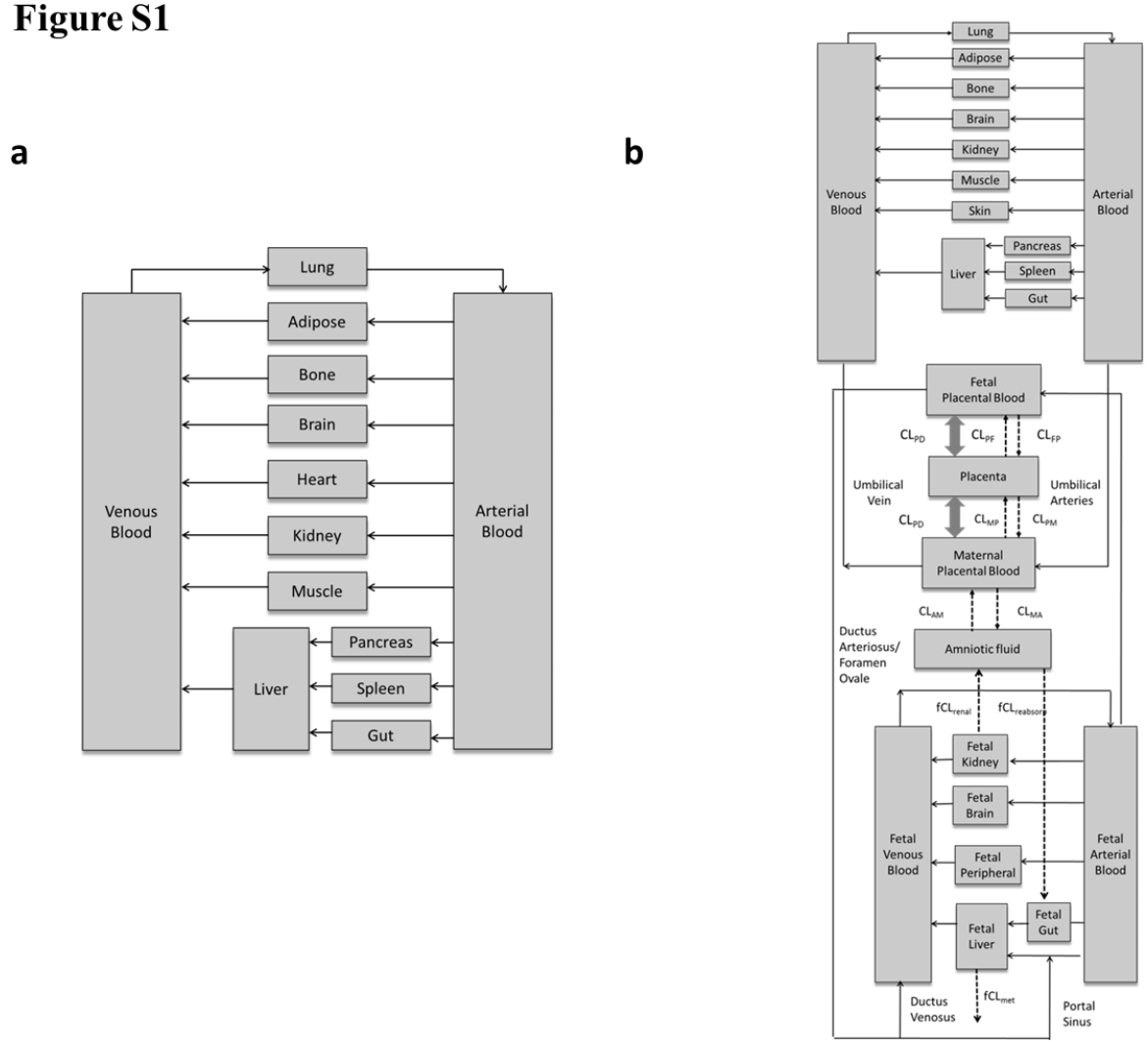


Figure S1: a shows the structure of the MATLAB PBPK model for the non-pregnant population. **b** is the schematic diagram of the maternal-fetal full PBPK model. Solid arrows indicate tissue blood flows and dashed arrows indicate clearances. CL: clearance; Prefixes: f- fetal; Subscripts: PD- passive diffusion, M- maternal, P- placenta, F- fetus, A- amniotic fluid, met- metabolism, renal-renal excretion, reabsorp- amniotic fluid swallowing. $CL_{FP/PF}$ and $CL_{MP/PM}$ represent the unidirectional placental transporter-mediated clearances between the fetus and the placenta and between the mother and the placenta.

Figure S2

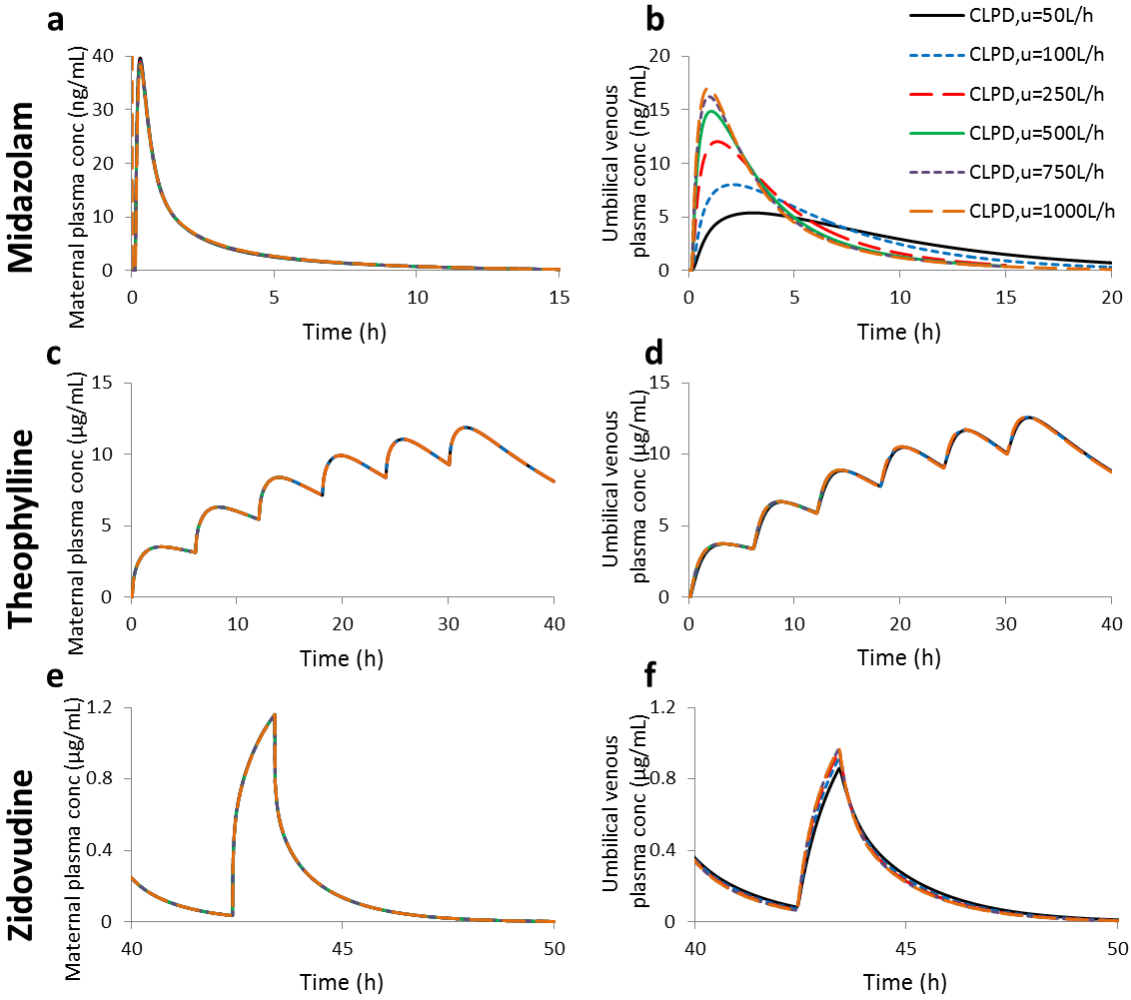


Figure S2: Impact of varying the unbound transplacental passive diffusion clearance ($CL_{PD,u}$)of midazolam, theophylline, and zidovudine on their maternal plasma (**a**, **c**, and **e** ,respectively) and umbilical venous plasma (**b**, **d**, and **e**, respectively) concentration-time (C-T) profiles. Variations in $CL_{PD,u}$ resulted in virtually no changes in the predicted maternal plasma drug concentrations for all three drugs. In the lower $CL_{PD,u}$ range (<250 L/h), varying $CL_{PD,u}$ resulted in changes in umbilical venous plasma midazolam C-T curves. However, these changes diminished as $CL_{PD,u}$ was further increased. In contrast, within the tested range (50-1000L/h), changes in $CL_{PD,u}$ resulted in negligible changes in umbilical venous plasma C-T profiles of theophylline or zidovudine.

Table S1: Sensitivity analysis of midazolam transplacental passive diffusion clearance ($CL_{PD,MDZ}$)

time	Observed UV plasma conc.	CL_{PD} (L/h)*											
		2.27		4.54		11.35		22.7		34.1		45.4	
		Pred	PE	Pred	PE	Pred	PE	Pred	PE	Pred	PE	Pred	PE
h	ng/mL	ng/mL	%	ng/mL	%	ng/mL	%	ng/mL	%	ng/mL	%	ng/mL	%
0.33	6	0.72	-88.03	1.89	-68.42	4.87	-18.85	7.83	30.49	9.25	54.19	10.22	70.39
0.33	9	0.72	-92.02	1.89	-78.95	4.87	-45.90	7.83	-13	9.25	2.79	10.22	13.59
0.45	4	1.45	-63.63	3.45	-13.65	7.72	92.89	11.51	187.75	13.16	228.98	14.29	257.23
0.75	29	3.14	-89.16	6.29	-78.31	11.76	-59.45	15.98	-44.91	17.57	-39.42	18.63	-35.77
0.83	7	3.51	-49.89	6.80	-2.83	12.34	76.29	16.46	135.16	17.96	156.61	18.95	170.73
0.83	19	3.51	-81.54	6.80	-64.20	12.34	-35.05	16.46	-13.36	17.96	-5.46	18.95	-0.26
1	18	4.15	-76.93	7.63	-57.61	13.12	-27.09	16.89	-6.14	18.16	0.91	18.97	5.40
Prediction precision		77.31		51.99		50.79		61.55		69.77		79.05	
Prediction bias		-77.31		-51.99		-2.45		39.43		56.94		68.76	
Sum of squares		1215.41		839.68		426.04		327.92		346.53		376.57	

* term midazolam unbound fraction increases from 0.3 to 0.454 as a result of hemodilution caused by pregnancy.

Supplementary Methods

Ordinary Differential Equations Defining the Mass Balance in the Fetal-PBPK

I. Symbols representing the variables and parameters used in the models

V	volume (L)
C	concentration (μM)
(t)	gestational age-dependent
Q	blood flow rate (L/h)
K_p	tissue: plasma partition coefficient
BP	blood: plasma partition ratio
CL	clearance (L/h)

Subscriptions:

subscription a	arterial
subscription F:M	from fetal to maternal
subscription M:F	from maternal to fetal
subscription A:M	from amniotic fluid to maternal
subscription M:A	from maternal to amniotic fluid
subscription af	amniotic fluid
subscription FP	from fetal placental blood to placenta
subscription PF	from placental to fetal placental blood

subscription p plasma

subscription PD passive diffusion

subscription pl placenta

subscription PM from placental to maternal placental blood

subscription MP from maternal placental blood to placenta

subscription PD passive diffusion

subscription af amniotic fluid

subscription gut gut

subscription kid kidneys

subscription liv liver

subscription per peripheral

subscription bra brain

subscription pl placenta

Superscriptions

superscription m maternal

superscription f fetal

superscription s placental

II Equations

Maternal placental blood

The mass balance in the maternal placental blood compartment can be described using the following differential equation Eq. 1

$$\begin{aligned} V_{pl}^{m}(t)\frac{d\left[C_{pl}^{m}\right]}{dt} &= Q_{pl}^{m}(t)(C_{a}^{m}-C_{pl}^{m}) \\ &+ CL_{PM}\ fu_{pl}(t)C_{pl} - CL_{MP}\frac{fu^{m}(t)}{BP^{m}(t)}C_{pl}^{m} \\ &+ CL_{AM}\ fu_{af}^{f}(t)C_{af}^{f} - CL_{MA}\frac{fu^{m}(t)}{BP(t)}C_{pl}^{m} \\ &+ CL_{PDPM}\left(fu_{pl}^{s}(t)\frac{1}{\beta_{pl}^{s}}C_{pl}^{s} - \frac{fu^{m}(t)}{BP^{m}(t)}\frac{1}{\alpha^{m}}C_{pl}^{m}\right) \end{aligned} \quad (1)$$

where C_a^m is the artieral blood concentration in the mother.

Placenta

The mass balance in the placental membrane compartment can be described using the following differential equation Eq. 2

$$\begin{aligned} V_{pl}^{s}(t)\frac{d\left[C_{pl}^{s}\right]}{dt} &= CL_{FP}\frac{fu^{f}(t)}{BP^{f}(t)}C_{pl}^{f} - CL_{PF}\ fu_{pl}^{s}(t)C_{pl}^{s} \\ &+ CL_{MP}\frac{fu^{m}(t)}{BP^{m}(t)}C_{pl}^{m} - CL_{SP}\,fu_{pl}^{s}(t)C_{pl}^{s} \\ &+ CL_{PDPF}\left(\frac{fu^{f}(t)}{BP^{f}(t)}\frac{1}{\alpha^{f}}C_{pl}^{f} - fu_{pl}^{s}(t)\frac{1}{\beta_{pl}^{s}}C_{pl}^{s}\right) \\ &+ CL_{PDPM}\left(\frac{fu^{m}(t)}{BP^{m}(t)}\frac{1}{\alpha^{m}}C_{pl}^{m} - fu_{pl}^{s}(t)\frac{1}{\beta_{pl}^{s}}C_{pl}^{s}\right) \\ &- CL_{p0}C_{pl}^{s} \end{aligned} \quad (2)$$

where CL_{p0} is the irreversible placental metabolism

The fetal unit comprises of the fetal placental blood, amniotic fluid, the fetal circulatory system, fetal liver, fetal gut, fetal kidneys, fetal brian, and a lumped peripheral compartment representing the rest of the fetus.

The following equation is used to describe the concentration in the placenta/fetal compartment:

$$V_{pl}^{f}(t)\frac{d\left[C_{pl}^{f}\right]}{dt} = Q_{pl}^{f}(t)(C_{a}^{f} - C_{pl}^{f}) + CL_{PF}\, fu_{pl}^{s}(t)C_{pl}^{s} - CL_{FP}\frac{fu^{f}(t)}{BP^{f}(t)}C_{pl}^{f} + CL_{PDPF}\left(fu_{pl}^{s}(t)\frac{1}{\beta_{pl}^{s}}C_{pl}^{s} - \frac{fu^{f}(t)}{BP^{f}(t)}\frac{1}{\alpha^{f}}C_{pl}^{f}\right) \quad (3)$$

Amniotic fluid

$$V_{af}^{f}(t)\frac{d\left[C_{af}^{f}\right]}{dt} = CL_{MA}\frac{fu_{pl}^{m}(t)}{BP^{m}(t)}C_{pl}^{m} - CL_{AM}\, fu_{af}^{f}(t)C_{af}^{f} + \frac{CL_{renal}^{f}(t)}{BP^{f}(t)}C_{v}^{f} - CL_{reabs}^{f}C_{af}^{f} \quad (4)$$

where CL_{renal} is the renal clearance based on the fetal venous plasma concentration (please see the following Eq.5 for fetal kidneys and Eq. 11for fetal central venous blood compartment).

Fetal kidneys

$$V_{kid}^{f}(t)\frac{d\left[C_{kid}^{f}\right]}{dt} = Q_{kid}^{f}(t)(C_{a}^{f} - \frac{C_{kid}^{f}}{Kp_{kid}^{f}(t)/BP^{f}(t)}) \quad (5)$$

Note the renal excretion has been considered in the previous equation (4), as well as in equation (11), and not directly from this kidney compartment.

Fetal guts

The following equation is used to describe the concentration in the gut:

$$V_{gut}^{f}(t)\frac{d\left[C_{gut}^{f}\right]}{dt} = Q_{gut}^{f}(t)\left(C_{a}^{f} - \frac{C_{gut}^{f}}{Kp_{gut}^{f}(t)\Big/BP^{f}(t)}\right) + CL_{reabs}^{f}C_{af}^{f} \tag{6}$$

Fetal liver

The fetal liver is a well-stirred perfusion-limited organ, consisting of the tissue and its capillary blood. On the basis of well-stirred model, the emergent concentration is the driving concentration for hepatic metabolism.

Therefore the following equation is used to describe the concentration of liver compartment:

$$\begin{aligned} V_{liv}^{f}(t)\frac{d\left[C_{liv}^{f}\right]}{dt} = {} & Q_{port\_a}^{f}(t)C_{a}^{f} \\ & + Q_{port\_v}^{f}(t)C_{pl}^{f} \\ & + Q_{gut}^{f}(t)\frac{C_{gut}^{f}}{Kp_{gut}^{f}(t)\Big/BP^{f}(t)} \\ & - \left(Q_{hep\_ven}^{f}(t) + Q_{hep\_art}^{f}(t) + Q_{gut}^{f}(t)\right)\frac{C_{liv}^{f}}{Kp_{liv}^{f}(t)\Big/BP^{f}(t)} \\ & - fu^{f}(t)\Big/BP^{f}(t)\cdot CL_{f0}\cdot\frac{C_{liv}^{f}}{Kp_{liv}^{f}(t)\Big/BP^{f}(t)} \end{aligned} \tag{7}$$

where CL_{f0} is the fetal hepatic metabolic clearance and subscription hep_ven and hep_art and gut refer to the hepatic blood supplied by umblical vein (minus ductus venosus blood flow) and by the hepatic artery, respectively. The former is from the fetal placental blood compartment (Figure 1).

Fetal peripheral compartment

$$V_{per}^{f}(t)\frac{d\left[C_{per}^{f}\right]}{dt} = Q_{per}^{f}(t)\left(C_{a}^{f} - \frac{C_{per}^{f}}{Kp_{per}^{f}(t) \Big/ BP^{f}(t)}\right) \tag{8}$$

Fetal brain

$$V_{bra}^{f}(t)\frac{d\left[C_{bra}^{f}\right]}{dt} = Q_{bra}^{f}(t)\left(C_{a}^{f} - \frac{C_{bra}^{f}}{Kp_{bra}^{f}(t) \Big/ BP^{f}(t)}\right) \tag{9}$$

where subscription bra means brain compartment.

Fetal central arterial blood compartment

$$V_{a}^{f}(t)\frac{d\left[C_{a}^{f}\right]}{dt} = \left(Q_{pl}^{f}(t) + Q_{kid}^{f}(t) + Q_{gut}^{f}(t) + Q_{port\_a}^{f}(t) + Q_{per}^{f}(t) + Q_{bra}^{f}(t)\right)\left(C_{v}^{f} - C_{a}^{f}\right) \tag{10}$$

Fetal central venous blood compartment

$$
\begin{aligned}
V_v^f(t)\frac{d\left[C_v^f\right]}{dt} = {} & (Q_{pl}^f(t) - Q_{hep\_ven}^f(t))C_{pl}^f \\
& - (Q_{pl}^f(t) + Q_{kid}^f(t) + Q_{gut}^f(t) + Q_{hep\_art}^f(t) + Q_{per}^f(t) + Q_{bra}^f(t))C_v^f \\
& + Q_{kid}^f(t)\frac{C_{kid}^f}{Kp_{kid}^f(t)\big/BP^f(t)} \\
& + Q_{per}^f(t)\frac{C_{per}^f}{Kp_{per}^f(t)\big/BP^f(t)} \\
& + Q_{bra}^f(t)\frac{C_{bra}^f}{Kp_{bra}^f(t)\big/BP^f(t)} \\
& + (Q_{gut}^f(t) + Q_{port\_a}^f(t) + Q_{port\_v}^f(t))\frac{C_{liv}^f}{Kp_{liv}^f(t)\big/BP^f(t)} \\
& - \frac{CL_{renal}^f(t)}{BP^f(t)}C_v^f
\end{aligned}
\tag{11}
$$