Research Article

A Simplified PBPK Modeling Approach for Prediction of Pharmacokinetics of Four Primarily Renally Excreted and CYP3A Metabolized Compounds During Pregnancy

Binfeng Xia,¹ Tycho Heimbach,^{1,4} Rakesh Gollen,² Charvi Nanavati,³ and Handan He¹

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Abstract. During pregnancy, a drug's pharmacokinetics may be altered and hence anticipation of potential systemic exposure changes is highly desirable. Physiologically based pharmacokinetics (PBPK) models have recently been used to influence clinical trial design or to facilitate regulatory interactions. Ideally, whole-body PBPK models can be used to predict a drug's systemic exposure in pregnant women based on major physiological changes which can impact drug clearance (*i.e.*, in the kidney and liver) and distribution (*i.e.*, adipose and fetoplacental unit). We described a simple and readily implementable multitissue/organ whole-body PBPK model with key pregnancy-related physiological parameters to characterize the PK of reference drugs (metformin, digoxin, midazolam, and emtricitabine) in pregnant women compared with the PK in nonpregnant or postpartum (PP) women. Physiological data related to changes in maternal body weight, tissue volume, cardiac output, renal function, blood flows, and cytochrome P450 activity were collected from the literature and incorporated into the structural PBPK model that describes HV or PP women PK data. Subsequently, the changes in exposure (area under the curve (AUC) and maximum concentration ($C_{\max}$)) in pregnant women were simulated. Model-simulated PK profiles were overall in agreement with observed data. The prediction fold error for $C_{\max}$ and AUC ratio (pregnant vs. nonpregnant) was less than 1.3-fold, indicating that the pregnant PBPK model is useful. The utilization of this simplified model in drug development may aid in designing clinical studies to identify potential exposure changes in pregnant women *a priori* for compounds which are mainly eliminated renally or metabolized by CYP3A4.

KEY WORDS: PBPK models; pharmacokinetics; physiological changes; pregnancy; systemic exposure.

INTRODUCTION

As the use of medications during pregnancy is common (1–3), efforts continue to focus on safe and efficacious dosing regimen allowing women and their doctors to make informed decisions about continued treatments during pregnancy. While some drugs are known to show altered pharmacokinetics during pregnancy (4), anticipation of systemic exposure

for medications taken by pregnant women has been difficult to determine, in part due to the lack of readily available whole-body physiologically based pharmacokinetics (PBPK) models. PBPK models have become more popular with both pharmacokinetic and formulation scientists, and advantages for the application of PBPK models as tools have been described (5,6), along with their structure, methodology, and implication in drug development (7–10). During pregnancy, apart from the development of the placenta and fetus, anatomical and physiological changes occur such as changes in blood flow, cardiac output, protein binding, renal, and hepatic functions (11,12); all of which may have significant effect on the pharmacokinetics (PK) and pharmacodynamics (PD) of drugs. A number of recently published pregnant PBPK models (p-PBPK) incorporate comprehensive information related to anatomical, physiological, and metabolic changes which required complex and resource-intensive parameter inputs and custom programming (13,14). Although these models account for various time-variant parameter changes during gestation, key factors that influence the pharmacokinetics for pregnant women have only been partially described. Moreover, it is not always practical for

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¹ Novartis Institutes for Biomedical Research, DMPK–Translational Sciences, One Health Plaza 436/3253, East Hanover, New Jersey 07470, USA.

² Division of Pharmaceutical Sciences, Pharmacy and Health Sciences, Long Island University, Brooklyn, New York, USA.

³ Department of Pharmaceutical Sciences, University at Buffalo, State University of New York, Buffalo, New York, USA.

⁴ To whom correspondence should be addressed. (e-mail: tycho.heimbach@novartis.com)

clinical or pharmaceutical researchers to develop highly complex PBPK models in limited timeframes. Here, we describe an easily implementable mechanistic PBPK model that can characterize the disposition of drugs in pregnant women for four primarily renally excreted and CYP3A-metabolized compounds.

Significant changes in PK are more likely to be observed for drugs which have single elimination pathways. It is known that the CYP450 pathway involved is affected by pregnancy (15). Hence, reference drugs selected were compounds which are (a) primarily excreted *via* kidney, (b) known substrates of CYP450 isoenzymes and which undergo phase II metabolic pathways (*e.g.*, *N*-acetyltransferase and glucuronidation, *etc.* (16)). Due to the regulations of the Food and Drug Administration, Clinical PK trials typically exclude both pregnant women and those of reproductive age because of ethical and practical considerations (17). For this reason, often only limited data are available for altered systemic PK parameters such as volume of distribution (V_d) and clearance (CL) change during pregnancy (17). Metformin, digoxin, midazolam, and emtricitabine were chosen as study drugs as published data were available (18–20). For metformin, digoxin, and emtricitabine, the major route of elimination is renal excretion. Midazolam is primarily metabolized and eliminated by CYP3A, which allows incorporating a specific-metabolizing enzyme change in the PBPK model. To our current knowledge, p-PBPK models have not been extensively described for prediction and analysis of PK changes for renally excreted compounds during pregnancy. Midazolam p-PBPK model has been established by Gaohua using Matlab Simulink. However, C_{max} , area under the curve (AUC), and distribution phase were underpredicted (13). Here, we applied a simplified p-PBPK modeling strategy to predict the PK profiles in pregnant women by considering the essential physiologic and metabolic changes that occur during pregnancy for metformin, digoxin, midazolam, and emtricitabine. Furthermore, the simulated PK profiles were compared against published PK profiles from clinical outcomes (examples from literature) for model verification.

MATERIALS AND METHODS

Computer Hardware and Software

GastroPlus (version 8.0, Simulations Plus, Inc, CA, USA) embedded with the Advanced Compartmental Absorption Transit (ACAT) model and PBPKPlus™ module was run on a Lenovo computer with Intel® Core™ i5 processor. The ACAT model, originally established by Yu and Amidon (21), enables the prediction of rate and extent of oral drug absorption. The PBPKPlus™ module incorporates a whole-body PBPK model which defines mathematical compartments for all major tissues using a group of differential equations (22). This module can further predict the amount of drug that distributes to each tissue as a function of time as well as the PK profiles using the input parameters of physicochemical properties (*e.g.*, solubility, permeability, LogP, pK_a , and particle sizes) and disposition data (*e.g.*, tissue-to-plasma partition coefficients (K_p), CL, and first-pass extraction ratio (FPE)).

Development and Verification of PBPK Model in Pregnant Women

General Strategy

The present work simulated the PK of four model drugs in nonpregnant or pregnant women using PBPK models comprising of 14 tissue/organ compartments (Fig. 1). Transport of drug from systemic circulation into tissues was assumed to be perfusion rate limited. Within the default GastroPlus PBPKPlus module, the reproductive organ, renamed as fetoplacental units, was used to represent lumped intra-uterine components, including the fetus, placenta, amniotic fluid, and uterus. The compartment of the fetoplacental units was also assumed to be perfusion rate limited. The p-PBPK model integrated the generic physiological parameters, physiochemical properties, ADME data, and information related to clinical trial design. The PK data of model drugs (metformin, digoxin, midazolam, and emtricitabine) for women during postpartum (PP) and pregnant periods are obtained and digitized from literature (18–20). In these clinical studies, most of subjects received treatment in the third trimester (>28 weeks) and PP (>6 weeks), except for metformin, in which some subjects also received drug during early stages (10–14 weeks) and mid stages (20–26 weeks) of pregnancy. We assumed that the physiology and pharmacokinetics in PP subjects are representative of healthy nonpregnant or prepregnant women because the physiological, anatomical, and biochemical conditions are generally considered to return to the basal level after a normal delivery. The general workflow for pregnant PBPK model development and validation is illustrated in Fig. 2. Briefly, the PBPK model was initially developed using

Fig. 1. Structure of pregnancy physiologically based pharmacokinetic (p-PBPK) model

Fig. 2. Schematic representation of the workflow of pregnancy PBPK model development and validation for compounds that are mainly renally eliminated or metabolized by CYP3A4

the physicochemical, biopharmaceutical, and pharmacokinetic parameters obtained from literatures or *in silico* prediction tools (e.g., ADMET predictor) in nonpregnant women population, and the model was further validated by comparing the simulated PK data with the observed clinical results. Population-dependent physiological parameters in human PBPK models were obtained using the Population Estimates for Age-Related Physiology™ module in GastroPlus v8, where the generic demographic and physiological parameters (e.g., age, tissue volume, and perfusion rate) for a normal American female were based on the National Health and Nutrition Examination Survey as described previously (22). If predicted PK profile and parameters were significantly deviant from the observed data, the model was refined by parameter optimization by fitting against the nonpregnant clinical data, which is a generic refinement strategy especially for drugs in development. In GastroPlus, an optimization module was generally used to estimate parameters of interest after successful data fitting. The model refinement strategy may not be limited to this and may be case-dependent (e.g., applying scaling factor to match the observed parameter values). As illustrated in Fig. 2, a simplified p-PBPK model (model 1) was initially developed with limited physiological (e.g., body weight (BW), cardiac output (CO), glomerular filtration rate (GFR), and renal secretion) and metabolic (e.g., CL changes contributed by CYP450 enzymes) changes. Alternatively, a comprehensive pregnant model (model 2) based on model 1 was extended by further considering the physiological changes (e.g., volume and perfusion rate) in fetoplacental unit, adipose tissue, and body fluid (e.g., volume of

blood). The volume and perfusion rate for each tissue were automatically calculated using direct model balancing or manually defined using the regression equations reported by Abduljalil *et al.* (14) for models 1 or 2, respectively. In addition, the gut physiology was assumed to be the same between nonpregnant and pregnant populations. The predicted mean PK parameters (C_{max} , AUC, and peak concentration time (t_{max})) for PP and pregnant women as well as the ratios of the mean PK parameters (pregnant vs. PP) were then obtained based on the simulations.

Development and Validation of PBPK Model in Nonpregnant Subjects

Physicochemical parameters were either obtained from literature or predicted by the GastroPlus built-in ADMET predictor. Key ADME-related parameters (e.g., solubility, effective permeability (P_{eff}), CL, and V_d) were obtained from the literature (see details in Electronic Supplementary Material (ESM) Table S1). The ACAT model was used to quantitatively describe multiple drug absorption processes, including release, dissolution, precipitation, and passage across the GI membrane. The default gastrointestinal physiology parameters (e.g., pH, volume, transit time, bile salt concentration, etc.) under fasted and fed conditions within the ACAT model were based on the average values obtained from published population data. Rapid partitioning equilibrium of drugs was assumed between a homogenous tissue and plasma/blood, and thus perfusion-limited distribution kinetics

was used for all the tissues in the PBPK model, K_p values were initially calculated using the tissue composition (TC) equations, according to the relationship between physiological data (lipids, plasma proteins and lipoproteins, and water *etc.*) and compound-specific determinants of distribution like lipophilicity (Log P), ionization (pK_a), and plasma protein binding (f_{up} ;23). Transporter-mediated tissue distribution was not considered in the current model. The steady state V_d (V_{ss}) was then estimated using Eq. (1)

$$V_{ss} = V_{\text{plasma}} + \sum V_{\text{tissue}} \times K_{p,\text{tissue}} \quad (1)$$

where V_{tissue} and V_{plasma} is the physiological volume for each tissue and plasma. Drug elimination was assumed to occur only in liver and/or kidney and was described using reported hepatic CL (CL_H), renal CL (CL_R), and GFR. If measured GFR values were not available from clinical studies, a generic recent value (114 mL/min) from the literature was used (14). The renal filtration clearance ($CL_{\text{filtration}}$) and net secretion clearance ($CL_{\text{secretion(net)}}$) were calculated using Eqs. (2) and (3), respectively.

$$CL_{\text{filtration}} = \text{GFR} \times f_{up}(\text{free drug fraction in plasma}) \quad (2)$$

$$CL_{\text{secretion(net)}} = CL_R - CL_{\text{filtration}} \quad (3)$$

The initial model was verified by comparing the simulated PK profiles with the observed data for nonpregnant population. The model was then refined, if needed, with the following approaches, depending on different scenarios. As described, the GastroPlus optimization module was utilized to estimate parameters that significantly influence PK profiles. Common deviations in the PBPK models were observed in that the predicted V_d using *in silico* TC equation was significantly different from the reported values. In general, simultaneous optimization of K_p for multiple tissues to fit against observed data are not feasible unless rich tissue distribution data are available. Here, we use two approaches to refine the PBPK model for better prediction. We adjusted one or multiple K_p values using experimental data obtained for nonpregnant population from the literature. Alternatively, if no experimental K_p values were available, the *in silico*-predicted K_p values of all tissues were uniformly multiplied by a scaling factor. The scaling factor was manually optimized until the V_d predicted by the adjusted model matches the observed values—such an optimization could be done with clinical data from First-In-Human or other nonpregnant studies. When the predicted absorption kinetics significantly deviated from observed kinetics, the reference solubility and P_{eff} were optimized using GastroPlus build-in optimization module™ to capture the absorption behavior. For the drug elimination, the input CL values were directly obtained from nonpregnant PK study (if CL is reported) or the mean (or median) values of the data range reported in literature. The detailed input parameters for four model compounds are summarized in ESM Table S1, and the K_p values used in the whole-body PBPK model have been summarized in Table I.

Physiological Changes Considered in the Pregnant PBPK Model

The disposition of many medications is altered during pregnancy due to considerable changes in the physiology, anatomy, and biochemistry. These changes alter the exposure (absorption, distribution, and elimination) of xenobiotics during and after pregnancy in women who receive similar doses. Recently, Abduljalil *et al.* summarized various mathematic algorithms to describe the dynamic changes of individual parameter values during pregnancy (14). These quantitative regression equations were also applied to adjust parameter input (*e.g.*, BW, tissue volume *etc.*) for the current PBPK modeling work (Table II).

Drug absorption may be altered as nausea and vomiting during early stage of pregnancy may result in delayed gastric emptying and reduced small intestine motility (16,24). Prolonged GI transit time can potentially increase the t_{max} but reduce the C_{max} (16). In addition, results have shown that there are no significant differences in basal gastric pH during pregnancy, compared with nonpregnancy values (14). However, gestation-induced alteration in absorption is clinically insignificant in most of the known case studies (25–27). Therefore, this was not considered in the current model. In the current PBPK model, the GastroPlus ACAT model (with default ASF optimized LogD v6.1) was used to describe drug absorption for nonpregnant and pregnant states.

For changes of volume of distribution, with the simplified pregnant PBPK model (model 1), drug distribution during pregnancy was assumed to be mainly impacted by changes of body weight and plasma protein levels. The average maximum weight gain during the pregnancy period is about 12.5 kg (28), which reflects an increase of total body water (~8 L) and adipose tissue (~4 kg) (15,16). Plasma protein levels, including albumin and α -acid glycoprotein, generally decrease during pregnancy relative to prepregnancy values. For example, plasma protein level gradually decreases since second trimester and eventually reach ~70 to 80% of the normal level at time of delivery (14,15,29). The fraction of f_{up} may increase due to decreased albumin or α -glycoprotein levels, which may increase drug distribution for drugs with high protein binding. However, the quantitative correlation between plasma level and f_{up} is not well understood. Therefore, f_{up} changes during pregnancy cannot be directly predicted based on plasma protein levels, and this is not considered in most of pregnant PBPK models (13,30). Here, f_{up} was assumed unaltered during pregnancy unless f_{up} was measured in pregnant women during the clinical trials (19). If needed, the K_p values were recalculated by the TC equations when plasma protein binding data were available. In the comprehensive pregnant PBPK model (model 2), more gestation-related changes and parameters were considered. A lumped fetal and placenta compartment (fetoplacental unit) was used to represent uterus, fetus, placenta, and amniotic fluid. Because substantial morphological and physiological changes in fetoplacental unit occur during pregnancy, the mass or volume of uterus, placenta, amniotic fluid, and fetus undergoes dramatic changes, which comprises about 35% of the total gestational weight gain during pregnancy (31). Increase of body fat may account for about 55% of total weight gain during pregnancy (32). During the course of pregnancy, plasma cell volume may increase 34–70% while

Table I. Tissue-to-Plasma Partition Coefficients (K_p) of the Model Drugs

Tissue	Metformin ^a	Digoxin ^b		Midazolam ^b		Emtricitabine ^a
		PP	Pregnancy	PP	Pregnancy	
Lung	1.53	9.36	8.95	0.31	0.33	3.95
Adipose	0.25	1.02	0.98	1.88	2.35	0.95
Muscle	1.73	35.7	35.7	0.59	0.72	3.75
Liver	1.64	10.83	10.34	0.95	1.16	3.8
Spleen	1.67	7.88	7.56	0.62	0.75	3.9
Heart	1.45	5.75	5.53	0.73	0.87	3.95
Brain	1.69	1.92	1.94	1.44	1.78	4
Kidney	1.55	11.78	11.24	0.64	0.77	3.9
Skin	1.23	3.61	3.50	0.9	1.06	3.6
Reproductive organ (fetoplacental unit)	1.55	11.78	11.24	0.66	0.76	3.9
Red bone marrow	0.95	2.0	2.0	1.66	2.03	2.25
Yellow bone marrow	0.25	1.02	0.98	1.88	2.35	0.95
Rest of body	1.67	7.88	7.56	0.65	0.78	3.75

^a The f_{up} of metformin and emtricitabine in plasma protein is assumed constant during pregnancy and PP since there are no data available to provide evidence that the f_{up} values change during pregnancy. Therefore, K_p values are not adjusted by f_{up} during pregnancy

^b The f_{up} values of digoxin and midazolam in plasma during pregnancy and PP (19) are available and thereby K_p values are adjusted by f_{up} during pregnancy

red blood cell volume increases to a lesser extent along with the plasma volume (14). Overall, the apparent V_d may increase during pregnancy, further resulting in lower systemic drug concentration when the unadjusted dose strength (dose used in drug labeling) is used in pregnant subjects. The change in the volume of these tissues during pregnancy can be described by using the equation summarized in Table II.

The change of metabolic enzyme activities, GFR, and renal blood flow rate can significantly alter the drug elimination during pregnancy. The metabolic activities of CYP3A4, CYP2D6, UGT1A1, and UGT1A4 are known to be induced by elevated estrogen/progesterone levels while the activities of CYP1A2 and CYP2C19 are known to be inhibited by progesterone and estradiol (12,16,33). For CYP3A metabolized compounds (e.g., midazolam), an average 35% increase of systemic CL and FPE in gut and liver were included in pregnant PBPK model across the gestational

ages (14–40 weeks) based on the findings by Tracy *et al.* (34). For really excreted compounds, the GFR and net secretion CL can increase up to 40% and 50% during pregnancy, respectively, resulting in enhanced renal excretion of unchanged drugs (16). Based on available data, GFR gradually increases during first 20–26 weeks of gestation and then reaches a plateau or slightly declines in the late stages of pregnancy (14,35). The increase in GFR in a particular gestation week was quantitatively estimated for the current pregnant PBPK model by equations reported by Abduljalil *et al.* (14). The dynamic changes of net renal secretion during pregnancy have not been well characterized. The net secretion clearance significantly increases during mid and late pregnancy, while is unaltered in early pregnancy (<12 weeks) as in the case of metformin (18). This finding also explains the ~40% increase in metformin net secretion clearance in mid and late pregnancy (>22 weeks), which was considered in current PBPK model. On the other

Table II. Main Anatomical, Physiological, and Biological Parameters Needed for p-PBPK Model and Equations Used to Calculate Parameter Values During Different Gestation Ages (GA) (14)

Parameter	Unit	Equation
Today body weight (kg)	kg	$TBW = 61.1 + 0.2409 \text{ GA} + 0.0038 \text{ GA}^2$
Cardiac output (CO)	L/h	$CO = 301 + 5.916 \text{ GA} - 0.088 \text{ GA}^2$
Total body fat mass (TFM)	kg	$TFM = 17.14 + 0.1305 \text{ GA} + 0.0008 \text{ GA}^2$
Weight of the uterus	g	$\text{Weight of the uterus} = 80 + 8.2931 \text{ GA} + 0.3546 \text{ GA}^2$
Fetal volume	mL	$\text{Fetal volume} = 0.01 \exp(13.604(1 - \exp(-0.0702\text{GA})))$
Placental volume	mL	$\text{Placenta volume} = 0.0 - 0.0716 + 0.9146 \text{ GA}^2 - 0.0122 \text{ GA}^2$
Amniotic fluid	mL	$\text{Amniotic fluid volume} = 0 + 1.9648 \text{ GA} - 1.2056 \text{ GA}^2 + 0.2064 \text{ GA}^3 - 0.0061 \text{ GA}^4 + 0.00005 \text{ GA}^5$
Volume of fetoplacental unit	mL	$\text{Fetoplacental volume} = \text{Uterus weight} + \text{Placenta volume} + \text{Fetal volume} + \text{Amniotic fluid volume}$
Blood flow of uterine	L/h	$\text{Uterine blood flow} = 1.71 + 0.2068 \text{ GA} + 0.0841 \text{ GA}^2 - 0.0015 \text{ GA}^3$
Plasma volume	L	$\text{Plasma volume} = 2.5 - 0.0223 \text{ GA} + 0.0042 \text{ GA}^2 - 0.00007 \text{ GA}^3$
Red blood cell (RBC) volume	L	$\text{RBC volume} = 1.49 + 0.0098 \text{ GA}$
Total blood volume	L	$\text{Total blood volume} = \text{plasma volume} + \text{RBC volume}$
Glomerular filtration rate (GFR)	mL/min	$\text{GFR} = 114 + 3.2367 \text{ GA} - 0.0572 \text{ GA}^2$

hand, the current model does not account for the change of net secretion clearance in early pregnancy (<14 weeks). In addition, there are no detailed literature data to describe the trend of secretion clearance changes between the 14 and 22 weeks of gestation. Therefore, the current PBPK model may not be suited to predict the PK of renally eliminated compounds for pregnant women during 14–22 weeks of gestation.

Cardiac output and uterine blood flow rate can significantly increase during pregnancy. The mean maternal cardiac output has a peak of ~7.30 L/min during the third trimester, which drops down to the baseline value during postpartum period (36,37). Uterine blood flow increases during pregnancy and peaks at late pregnancy as the uterus receives about 12% of cardiac output in comparison to 0.5% in prepregnant women. The longitudinal increase of cardiac output (models 1 and 2) and uterine blood flows (model 2 only) during pregnancy were calculated using polynomial regression equations in Table II.

Case Examples

Case Example 1: Metformin (BDDCS Class 3)

Metformin is a basic compound (pK_a : 10.37 and 2.33), with molecular weight (MW) of 129.2 g/mol and high aqueous solubility (129.6 mg/mL). Metformin is mainly excreted unchanged in urine with minimal metabolism (38) and is also a substrate for organic cation transporters (OCTs) (18,39–42). Thus, metformin is categorized as a biopharmaceutical drug disposition classification system (BDDCS) class 3 compound (43). Metformin pharmacokinetics was studied in 37 pregnant subjects and 12 of the subjects studied were in ≥ 12 weeks PP. Both pregnant and PP subjects received 500 mg oral dose of metformin twice daily. An increase of total renal clearance, creatinine clearance, and net secretion clearance was observed in mid and late pregnancy *versus* PP. Significant changes of metformin exposure (C_{max} and AUC) were observed during mid and late pregnancy compared to PP whereas intersubject variation in PK parameters was high (18). GFR and renal clearance during nonpregnancy was assumed to be equal to the observed values obtained from clinical trial participants during PP (18), while the corresponding renal secretion clearance was calculated by Eqs. (2) and (3). Physiological changes (as ratios of pregnancy *vs.* nonpregnancy) and the change of drug disposition parameter were estimated based on the modeling strategy described above during early (10–14 weeks, assuming 12 weeks in average), mid (22–26 weeks, assuming 24 weeks in average), and late pregnancy (34–38 weeks, assuming 36 weeks in average; Tables III and IV). The ratios were used to adjust the input physiological parameters during pregnancy for models 1 and 2, respectively [see ESM Fig. S1 for detailed parameter values. Early and mid pregnancy parameters were generated similarly (data not shown)]. K_p values for each tissue compartments within the PBPK model were calculated using Rodgers and Rowland's method to estimate the V_d of metformin since Roger and Rowland's method tends to be more appropriate to predict K_p values for moderate-to-strong bases (Table I; 44). The plasma samples were reportedly collected at steady-state. Therefore, the simulations were performed for early, mid, late pregnancy, and PP until the drug concentrations reached steady-state.

Case Example 2: Digoxin (BDDCS Class 3)

Digoxin is a neutral compound which is predominantly excreted *via* the kidneys and is a known P-glycoprotein (P-gp) substrate (45,46). Digoxin is a BCS/BDDCS class 3 compound due to its moderate solubility (0.25 mg/mL at pH 7.0), low permeability of (P_{eff} , 1.02×10^{-4} cm/s), and poor metabolism. Digoxin pharmacokinetics were studied in 13 women who received a single 0.25 mg oral dose during trimester (28–32 weeks) and PP (6–10 weeks; 19). The renal clearance and net secretion clearance during PP were obtained from (19) and the filtration clearance and GFR were then backcalculated using Eqs. (2) and (3). Hepatic metabolism of digoxin is known to play a minor role (~30%) in drug elimination (ESM Table S1). Metabolism is reported to be mediated *via* multiple pathways including hydrolysis and oxidation conjugation but it is not likely dependent on CYP450 system (47). CL_H of digoxin in nonpregnant women was not directly reported and was calculated as 4.2 L/h using the following equation (Eq. 4)

$$CL_H = CL_{total} - CL_{renal} = \left(\text{dose} / AUC_{0-\infty} \right) \times F - CL_{renal} \quad (4)$$

where the total oral CL is calculated by the ratio of dose and $AUC_{0-\infty}$; $AUC_{0-\infty}$ (15.74 ng $\times$ h/mL) was calculated from the mean concentration *vs.* time curve *via* noncompartmental analysis using WinNonlin Phoenix v6.2 (Pharsight Corporation, Cary, NC, USA). The average bioavailability (F) of digoxin was assumed to be 70% (60–80% dependent on formulation; 48,49). Calculated CL_{total} and CL_H were 11.1 and 4.2 L/h, respectively (Table IV). Currently, information regarding the activity changes of non-CYP enzymes during pregnancy is not available. Therefore, hepatic clearance of digoxin during pregnancy was assumed to be same as that during nonpregnant period. Other physiological parameter changes (as ratios of pregnancy *vs.* nonpregnancy, Table III) during late pregnancy (assuming average gestation age is 30 weeks) were estimated based on the modeling strategy described above. The ratios were used to adjust the input physiological parameters during pregnancy for models 1 and 2, respectively (see ESM Fig. S2 for detailed parameter values). K_p values for the tissue compartments were initially calculated using Poulin and Theil's TC equation because this TC equation yields better results for prediction of neutral compounds (23). K_p values for muscle of digoxin was estimated to be 35.7, which was obtained from the reported tissue binding data in human skeletal muscle (50). PK simulations were performed for women during late pregnancy and PP after a single oral dose (0.25 mg).

Case Example 3: Midazolam (BDDCS Class 1)

Midazolam (MW of 325.8 g/mol) is a BCS/BDDCS class 1 compounds with high aqueous solubility and high permeability (P_{eff} , 6.28×10^{-4} cm/s). Midazolam is a sensitive CYP3A substrate with significant gut metabolism and hepatic extraction after oral dose (19,51–53). Midazolam pharmacokinetics were studied in 13 women who received an oral dose of 2 mg immediate release formulation during trimester (28–32 weeks) and PP (6–10 weeks) (19). Drug

Table III. Fold Change of Gestation Age-Dependent Parameters Used for Four Case Studies in the Developed p-PBPK Model

Gestation ages (week)	12 ^a	24 ^a	30 ^b	35.3 ^c	36 ^a
Body weight	1.06	1.13	1.17	1.22	1.22
Cardiac output	1.19	1.30	1.33	1.33	1.33
Volume of fetoplacental unit	5.60	25.67	43.57	61.15	63.43
Blood flow of fetoplacental unit	8.02	20.10	25.21	27.97	28.17
Volume of total body fat	1.09	1.18	1.23	1.27	1.27
Total blood Volume	1.08	1.29	1.38	1.43	1.43
GFR (mL/min)	1.27	1.39	1.40	1.38	1.37

^a 12, 24, and 36 weeks are assumed to be the average gestation weeks reported in metformin case study

^b 30 weeks is assumed to be the average gestation average gestation week for midazolam and digoxin

^c Median gestation week reported for emtricitabine case study

elimination of midazolam is known to be mediated mainly by CYP3A metabolism and the hepatic CL used in the current model was estimated as the average value of the CL values obtained from multiple study reports (ESM Table S1). K_p values in different compartments were calculated by Rodgers and Rowland's TC equation (54) and f_{up} was reported as 0.67 during PP but decreases to 0.63 during late pregnancy (19). Due to increased enzyme activity of CYP3A during pregnancy, hepatic CL and gut FPE were increased by 35% (Table IV; 34). The changes of physiological parameters (as ratios of pregnancy vs. nonpregnancy, Table III) during late pregnancy (average 30 weeks) were estimated based on the modeling strategy described above. The ratios were used to

adjust the input physiological parameters during pregnancy for models 1 and 2, respectively (see ESM Fig. S2 for detailed parameter values). PK simulations were performed for women during late pregnancy and PP.

Case Example 4: Emtricitabine (BDDCS Class 1)

Emtricitabine (MW: 247.3) is a weak basic drug with pK_a of 2.65, with high permeability and high solubility. The compound is mainly excreted through kidneys and is likely an OCT-2 substrate (45,46). It is a once-daily nucleoside reverse transcriptase inhibitor that selectively and potently inhibits human immunodeficiency virus type 1 (HIV-1) replication (55).

Table IV. Parameters for Model Drugs in Non-Pregnant and Pregnant PBPK Model. Parameter Values Used in the Pregnancy PBPK Model are in *Italics* (e.g., Nonpregnant Parameter Values→Late Pregnant parameter values)

Parameters	Metformin	Digoxin	Midazolam	Emtricitabine
Basic compound/formulation Information				
Dose (mg)	500	0.25	2	200
Dosing regimen	Twice daily	Single dose	Single dose	Once daily
Main clearance pathways	Renal	Renal (~60%)	Hepatic (CYP3A4)	Renal
Formulations	IR tablet	IR tablet ^a	IR tablet ^a	IR capsule ^a
Gut physiology	Fasted	Fasted ^a	Fasted ^a	Fasted ^a
Parameters not influenced by pregnancy (e.g., physicochemical properties)				
Acid/base	Base	Neutral	Base	Base
MW (g/mol)	129.2	781	325.8	247.3
Log D at pH 7.4	-1.386	-1.4	3.56	-0.43
pK_a	10.37	12.98	5.5	2.65
Solubility in water (mg/mL)	129	0.25	0.024	112
Effective permeability (10^{-4} cm/s)	0.25	1.02	6.28	2.00
Diffusion Coefficients (cm^2/s)	1.21	0.439	0.856	0.75
Blood-to-plasma ratio	0.7	1.1	0.55	1
BCS/BDDCS Class	3	3	1	1
Parameters influenced by pregnancy (e.g., disposition parameters)				
Fraction unbound in plasma (f_{up} , %)	100→100	63→67	0.61→0.71	96→96
V_{ss} predicted by PBPK (L)	82.7→101 ^b 100 ^c	643→872 ^b 845 ^c	85.8→125 ^b 120 ^c	156→210 ^b 191 ^c
Gut FPE (%)	0	0	40→54	0
Hepatic CL (L/h)	0	4.2→4.2	24.5→33.1	0
Renal CL (L/h)	28.5→40.0	6.9→10.2	N/A	19.1→26.6
GFR (L/h)	9.9→13.8	7.4→10.5	N/A	6.8→9.4
Filtration CL (L/h)	9.9→13.8	4.6→7.01	N/A	6.6→9.1
Secretion CL (L/h)	18.7→26.2	2.3→3.11	N/A	12.5→17.6

^a The corresponding information is not available and was assumed

^b V_{ss} predicted by model 1

^c V_{ss} predicted by model 2

Emtricitabine is commonly used in HIV-1 infected women during pregnancy. Emtricitabine pharmacokinetics were studied in 26 women who received an oral dose of 2 mg immediate release formulation during trimester (median, 35 weeks) and PP (median, 8 weeks). Clinical PK data were digitized from literature (20) for women during third trimester and PP women after 4 days of 200 mg oral doses given once daily, and the oral CL (CL/F) and absolute oral bioavailability was observed as 20.6 L/h and 93%, respectively. Therefore, in the current model, the systemic CL of emtricitabine for healthy nonpregnant women was estimated to be 19.1 L/h. The generic GFR values (114 mL/min or 6.84 L/h) of healthy nonpregnant women were used in the model (14). The corresponding renal net secretion clearance (12.5 L/h) was calculated by Eqs. (2) and (3). Changes of physiological parameters (as ratios of pregnancy vs. nonpregnancy, Table III) during late pregnancy (average 35 weeks) were estimated based on the modeling strategy described. The ratios were used to adjust input physiological parameters during pregnancy for models 1 and 2, respectively (see ESM Fig. S3 for detailed parameter values). PK simulation for emtricitabine was performed for women during late pregnancy and PP based on the parameter input provided in Table IV.

RESULTS

Case Example 1: Metformin

The simulated plasma concentration vs. time profiles based on models 1 and 2 both captured the observed PK data during late pregnancy and PP (Fig. 3a) with regression coefficients (R^2) greater than 0.91. There were no significant differences in simulated results between models 1 and 2. The predicted AUC and C_{max} and the fold changes of these PK parameters (pregnant vs. PP) were within 1.1-fold of observed values (Table V). The model predicted renal clearance (CL_r , 39.98 L/h) during third trimesters is in agreement with observed clearance (37.5 L/h). The predicted mean fold change of AUC (pregnant vs. nonpregnant) was 0.69 and 0.71 based on the simulation using models 1 and 2, respectively, which is very close to the observed mean fold change (0.70). Although the predicted t_{max} was slightly longer than the observed values, the current PBPK model accurately predicts the PK of metformin during late pregnancy.

In addition, the simulated plasma concentration vs. time profiles based on models 1 and 2 were able to capture metformin's elimination profiles during early and mid pregnancy (Fig. 4). The R^2 of the simulation are higher than 0.78 and 0.86 for early and mid pregnancy, respectively. However, the distribution phase was slightly overpredicted in early pregnancy group whereas the C_{max} was overpredicted (deviation, ~20%) in mid pregnancy group.

Case Example 2: Digoxin

The muscle K_p value (4.3) estimated by Poulin and Theil's TC equation was dramatically lower than the reported K_p values (35.7), which resulted in underprediction of terminal $t_{1/2}$ (predicted (11.3 h) vs. observed (46.5 h)). The model was refined with literature reported muscle K_p value and accurately simulate the terminal phase PK curve with a predicted $t_{1/2}$ of 43.5 h. The simulated plasma concentrations vs. time profiles were in agreement with the observed profiles (Fig. 3b) with R^2 of 0.81

and 0.90 for models 1 and 2, respectively. The predicted AUC and C_{max} and the fold changes of the PK parameters (pregnant vs. PP) were within 1.2-fold of observed values (Table V). The predicted renal clearance (CL_r , 10.4 L/h) for women during third trimesters was in agreement with observed data (10.9 L/h). The predicted fold change of C_{max} (pregnant vs. nonpregnant) were 0.83 and 0.80 using models 1 and 2, respectively, similar to observed ratio, while the predicted fold change of AUC (model 1, 0.84; model 2, 0.85) has no large difference with the observed values (0.81).

Case Example 3: Midazolam

The *ex vivo* measured unbound drug fraction in plasma is 0.0071 and 0.0061 in PP and pregnant women, which were incorporated in the midazolam PBPK models. Midazolam is a compound that undergoes extensive metabolism in liver and gut. The simulated plasma concentration vs. time profile captured the observed profile well (Fig. 3c) with an R^2 of 0.78 and 0.76 for models 1 and 2, respectively. The predicted AUC and C_{max} were within 1.3-fold of observed values (Table V) using either models 1 or 2. The simulated ratio of C_{max} (pregnant vs. PP=0.54–0.56) was slightly underpredicted, compared to the observed values (0.72). On the contrary, the ratio of AUC was accurately predicted by the model (predicted (0.54–0.55) vs. observed (0.54)) (Fig. 4). The model successfully estimated the terminal phase half-life (predicted, 2.5 h; observed, 2.4 h). The PBPK model also predicted the short t_{max} and rapid absorption of midazolam. C_{max} prediction for pregnant women was improved (prediction fold error, 1.2) compared with the model reported previously (13) which underpredicted C_{max} by ~40%. The prediction fold error of AUC during pregnancy using the current PBPK model was 1.3 regardless of the type of model, which was similar to the fold error of 1.35 (observed AUC of 9.5 ng h/mL vs. predicted AUC of 7.05 ng-h/mL) predicted by a recently described midazolam p-PBPK model (13), indicating that the current PBPK model exhibits good prediction accuracy for pregnant women. In addition, there were no significant differences in simulated results between models 1 and 2.

Case Example 4: Emtricitabine

Oral V_d/F reported by the literature is about 4 L/kg (20) and the bioavailability with an IR capsule formulation is reported to be 93%, suggesting that the systemic V_{ss} is about 3.7 L/kg. The Rodger and Rowland's TC equation based on the input physicochemical parameters dramatically underpredicted V_{ss} (0.60 L/kg). To our best knowledge, no observed tissue distribution data are available. Therefore, the K_p values in all tissues were multiplied by a scaling factor of 5 (based on nonpregnant data) and the simulated PK profiles fit to the observed data (Fig. 3d) with R^2 of 0.80 and 0.84 for models 1 and 2, respectively. Predicted AUC and C_{max} were 1.0- and 1.5-fold of observed clinical data (Table V), respectively. The predicted fold changes of AUC and C_{max} (pregnant vs. nonpregnant) were within 1.3-fold of the observed ratios. The model predicted renal clearance (CL_r , 26.6 L/h) during third trimesters was in agreement with observed oral clearance (25.3 L/h). Therefore, the emtricitabine pregnant PBPK model is well verified and can well predict the alternation of PK during late pregnancy. There

Fig. 3. Simulated and observed mean plasma concentration in PP and late pregnant women for **a** metformin after 500 mg twice-daily oral dosing for 3 days, **b** digoxin following a single oral 0.25 mg dose, **c** midazolam after a single 2 mg oral dose, and **d** emtricitabine after once-daily 200 mg oral dose for 4 days

were no significant differences in simulated results between models 1 and 2.

DISCUSSION

Ideally, the use of pharmacologic agents would be minimized for pregnant women. However, pregnant women with certain medical conditions may still require ongoing treatment. Clinical trials actively exclude pregnant women due to ethical and scientific reasons. Therefore, human PK data are seldom collected during pregnancy for appropriate dosage and frequency of administration. Even after years of marketing, product labels regarding PK and dose adjustments often provide limited information for pregnant patients. The US Food and Drug Administration and European Medicine Agency have required that application sponsors collect and maintain data on the effects of prescription medicines that are used by pregnant women with a new labeling system. Thus, regulatory agencies encourage drug companies to anticipate and predict drug exposure during pregnancy prospectively. Physiological changes during pregnancy can alter drug disposition according to clinical data reported in the literature. Currently, optimization of dosing regimen for pregnant women can be challenging, especially in the absence of clinical data. PBPK has been recently recognized as useful tool to provide

valuable insights to aid clinical design. With incorporation of gestation-induced physiological changes, the pregnancy PBPK model can serve as a feasible alternative to empirical dose adjustment and guide the clinical trial design in pregnant women.

While PBPK models now have increased impact and applications in the pharmaceutical industry, the limitations in PBPK modeling need to be acknowledged and understood. In particular, K_p values can be inaccurately estimated using the *in silico* equations, resulting in biased prediction of V_d (56). If *in vivo* distribution data are not available in such situations, PBPK models cannot be readily established with high confidence. However, in some cases, if the PK profile of the compounds is characterized by a mono-exponential equation (similar to a one-compartmental model), the application of a uniform scaling factor on each K_p value likely provides good estimations for V_d and the shape of PK profiles as in the described case study of emtricitabine. The deviation in K_p predictions using *in silico* equations can be safely assumed to be similar across all tissues and can be corrected by applying a scaling factor to all K_p predicted by TC equations, if drug distribution can reach equilibrium rapidly (as with the assumption of a one-compartmental PK model). However, if the PK profile is described by a complicated multi-exponential equation, such assumptions may not stand because the magnitude of deviation of K_p prediction can differ between tissues (*e.g.*, digoxin). In such cases, the PBPK modeling approach may

Table V. Comparison Between Observed and PBPK Model Predicted Parameters

Drug	PK Parameters	Pregnant Women (third trimester)			Non-pregnant Women (PP)			Fold Change (pregnant vs. non-pregnant)		
		Observed ^a	Predicted ^b	Prediction Fold error ^c	Observed ^a	Predicted ^b	Prediction Fold error ^c	Observed ^a	Predicted ^b	Prediction Fold error ^c
Metformin	C_{max_ss} (ng/mL)	1,135	1,068, 1,070	1.0, 1.1	1,611	1,471	1.1	0.71	0.73, 0.73	1.0, 1.0
	t_{max} (h)	2.0	2.5, 2.6	1.2, 1.3	1.8	2.4	1.4			
	AUC_{12h_ss} (ng h/mL)	6,937	6,563, 6,698	1.0, 1.0	9,804	9,463	1.0	0.70	0.69, 0.71	1.0, 1.0
Digoxin	C_{max} (ng/mL)	0.8	0.84, 0.81	1.1, 1.0	1.1	1.01	1.1	0.90	0.83, 0.80	1.1, 1.1
	t_{max} (h)	1.6	1.7, 1.6	1.1, 1.0	1.3	1.6	1.2			
	AUC_{48h} (ng h/mL)	7.3	8.0, 8.1	1.1, 1.1	9.3	9.54	1.0	0.81	0.84, 0.85	1.0, 1.0
Midazolam	C_{max} (ng/mL)	6.4	5.33, 5.11	1.2, 1.3	9.3	9.5	1.1	0.72	0.56, 0.54	1.3, 1.3
	t_{max} (h)	0.56	0.4, 0.4	1.4, 1.4	0.54	0.4	1.4			
	AUC_{inf} (ng h/mL)	9.5	12.5, 12.2	1.3, 1.3	17.9	22.7	1.1	0.54	0.55, 0.54	1.0, 1.0
Emleritabine	C_{max_ss} (μg/mL)	1.4	0.94, 0.96	1.5, 1.5	1.4	1.3	1.2	0.97	0.72, 0.74	1.3, 1.3
	t_{max} (h)	2.0	1.4, 1.5	1.4, 1.3	2.0	1.3	1.4			
	AUC_{24h_ss} (μg h/mL)	8.0	8.0, 8.0	1.0, 1.0	9.7	11.2	1.1	0.82	0.71, 0.71	1.2, 1.2

^a Observed data was obtained from values reported in the literatures^b Predicted PK parameters are presented as “model 1, model 2”^c Fold error (FE) was calculated as $FE = 10^{\log(Predicted/Observed)}$. Calculated FE are presented as “model 1, model 2” **Fig. 4.** Simulated and observed mean plasma metformin concentration in early (a) and mid pregnancy (b) after 500 mg twice-daily oral dosing for 3 days

require additional data, such as drug tissue distribution data (e.g., quantitative whole-body autoradiography; 22).

Several pregnancy PBPK models have been introduced for xenobiotic disposition in pregnant women (57–59). However, few publications focused on the investigation of maternal PK alteration caused by the effect of pregnancy. Recently, Gaohua *et al.* developed a first pregnancy PBPK model incorporating time-dependent activity of cytochrome P450 enzymes (e.g., CYP1A2, CYP2D6, and CYP3A4) and implementing gestational time-dependent physiological changes (13). This pregnancy PBPK model successfully described the disposition for drugs which undergoes extensive metabolism by major cytochrome P450 enzymes. Although a dynamically parameterized pregnant PBPK model can predict the PK outcome throughout the pregnancy, model development, validation, and application may be time-consuming due to its complexity. In general, most PK/PD studies in pregnant women are conducted in third trimesters, with the baseline assessment for comparison to the pregnant state during the PP period. To this end, model development efforts, especially modeling validation, mainly target the periods of late pregnancy when PK/PD endpoints are most commonly collected in clinical studies. Maternal drug clearance is often altered due to changes in metabolic activity of enzymes, GFR, and renal secretory transporters. The change in enzyme activity (induction or inhibition) plays an important role in altering PK for many

drugs. Our practical and simplified PBPK model incorporates the change of essential parameters (*e.g.*, BW, CO, GFR, CL, *etc.*), and successfully estimated the PK of several compounds during late pregnant and PP periods. The fetal and placenta, adipose, plasma, and blood volume have dramatic changes during pregnancy, which are likely expected to exhibit significant effects on drug distribution. Therefore, these changes are considered and incorporated in model 2. Interestingly, the predicted results were very similar for four drugs using the simple model (model 1) and the comprehensive model (model 2). This suggests that the increase of drug volume of distribution (unit, liter) during pregnancy can be explained by total body weight gain and is nearly proportional to the body weight. The individual contribution of the tissues/organs, even with substantial gestation-induced physiological changes, may be less important if body weight changes have been considered by the p-PBPK model, unless compounds distribute much more extensively into these tissues than other tissues/organs without significant alteration. For example, all four compounds are expected to have no extensive distribution into adipose tissue ($K_p < 2$), and thereby the proportionality between volume of distribution and body weight is not altered by fat gain, as shown by the models 1 and 2 predicted V_{ss} values listed in Table IV. For tissue/organ gain with less composition of the total weight gain, such as fetoplacental unit which accounts for ~35% of total weight gain (65% for adipose tissue), their volume increase may have even less influence on PK. The current p-PBPK model can be easily implemented using conventional PBPK modeling tools (*e.g.*, GastroPlus or Simcyp), and should be sufficient to provide rational and accurate prediction for most of drugs which are mainly eliminated *via* the kidney or metabolized by CYP3A4 in the liver.

For renally excreted compounds, such as metformin, digoxin, and emtricitabine, the predicted fold decreases in AUC and C_{max} were comparable to the observed results. The simulated results suggested that the decrease of systemic exposure in these compounds were mainly attributed to an increased renal secretion and filtration during pregnancy. The increase of GFR in pregnancy is correlated to dilation of renal vasculature caused by the systemic vasodilation possibly mediated by progesterone and relaxin (35,60). Enhanced renal secretion CL has been reported for metformin, digoxin, amoxicillin, and certain endogenous compounds during pregnancy, suggesting of pregnancy-induced enhancement of expression and/or function of renal secretion transporter regulation. Within these three modeled compounds, namely metformin, digoxin, and emtricitabine, are known as the substrates for transporters expressed on apical or basolateral (luminal) membrane of the kidney proximal tubules. For example, OCT2 plays an important role in the renal clearance of metformin in humans (61). Similarly, basolateral renal transport of emtricitabine appears to proceed *via* OCT2 in S2 and/or S3 segments of the proximal tubule (62). Also, digoxin is well-known as the substrate of P-gp which transports drugs across the apical membrane of renal tubular epithelium (63–65). Although magnitude of enhanced activities for these transporters during pregnancy is not well-known, the alteration of net tubular secretion during pregnancy is considered in the current PBPK model by assuming 40% increase of $CL_{secretion}$ based on the previous evaluation in the pregnancy PK of metformin (18). Interestingly, the predicted and observed AUC and C_{max} fold changes are less than 30% in late pregnancy, compared to PP period. Accounting for the uncertainty caused by intersubject variability, the effect of

pregnancy on PK appears not be very large for drugs that are predominately eliminated *via* kidney.

The current PBPK modeling approach was further evaluated using midazolam which is extensively metabolized by CYP3A4. To reflect the change of CYP3A4 activity in third trimesters during pregnancy, the liver and gut metabolism, represented by hepatic CL and gut FPE, was increased by 30% according to previous clinical results. The prediction accuracy of the current model is similar to the PBPK model which integrates the time-dependent activity of CYP3A4. Since CYP3A4 plays the most important role in metabolism of drugs and xenobiotics, the current model can be used to anticipate the PK alteration and support drug labeling in many investigational drugs and postmarketing products for the pregnant populations. This also suggests that higher midazolam dose may be required in pregnant women to obtain the optimal pharmacological effect. In addition, alternation of activities in other metabolizing enzymes can be easily integrated in the pregnancy PBPK models using a similar approach. For example, it is known that the activities of CYP1A2 and CYP2C19 decreased by 30–65% (66) and 50% (67,68) whereas the activities of CYP2A6, CYP2D6, and UGT1A4 increased by 54% (69), 50% (70), and 200–300% (71) in late pregnant period, respectively.

The degree to which drugs are bound to the plasma proteins also influences their disposition. Albumin concentration is reported to fall by 20–30% during pregnancy, which may alter the drug concentration of albumin-bound compounds (15,29,72). Minor changes of f_{up} have been reported for midazolam and digoxin (19). Metformin and emtricitabine are negligibly bound to plasma proteins in human. Overall, the influence of alternation of plasma protein during pregnancy has minimal effects to the PK behaviors of our modeled compounds. In addition, the increased total body water/fat creates a larger space where the drugs may distribute. Therefore, minor changes in free drug concentration are expected as a result of the compensation effects caused by more extensive distribution of unbound drugs during pregnancy. Overall, there is little practical importance of plasma protein binding in the pharmacological response in pregnant patients, although the total drug concentration may alter for some drugs such as phenytoin.

CONCLUSIONS

A simplified p-PBPK model is described, which yields good predictions for renally cleared and CYP3A4 hepatically cleared compounds evaluated (metformin, digoxin, midazolam, and emtricitabine) when compared to clinical observations. The model is readily implemented in GastroPlus and could be applied for drugs with similar clearance pathways at various stages of drug development to evaluate if altered pharmacokinetics may occur in a pregnant population based on healthy volunteer data.

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