

A pregnancy physiologically based pharmacokinetic (p-PBPK) model for disposition of drugs metabolized by CYP1A2, CYP2D6 and CYP3A4

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Changes in physiology and biology during pregnancy lead to variation of the kinetics of drugs; therefore, a similar dose of a drug may not be associated with similar drug exposure to that found in nonpregnant women.

WHAT THIS STUDY ADDS

- This study introduces a novel physiologically based model, which integrates the knowledge of changes in various elements related to kinetics, particularly those of enzyme activity for three different cytochrome P450 enzymes, and shows the validity of assumptions on three cases related to caffeine, metoprolol and midazolam.

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AIMS

Pregnant women are usually not part of the traditional drug development programme. Pregnancy is associated with major biological and physiological changes that alter the pharmacokinetics (PK) of drugs. Prediction of the changes to drug exposure in this group of patients may help to prevent under- or overtreatment. We have used a pregnancy physiologically based pharmacokinetic (p-PBPK) model to assess the likely impact of pregnancy on three model compounds, namely caffeine, metoprolol and midazolam, based on the knowledge of their disposition in nonpregnant women and information from *in vitro* studies.

METHODS

A perfusion-limited form of a 13-compartment full-PBPK model (Simcyp® Simulator) was used for the nonpregnant women, and this was extended to the pregnant state by applying known changes to all model components (including the gestational related activity of specific cytochrome P450 enzymes) and through the addition of an extra compartment to represent the fetoplacental unit. The uterus and the mammary glands were grouped into the muscle compartment. The model was implemented in Matlab Simulink and validated using clinical observations.

RESULTS

The p-PBPK model predicted the PK changes of three model compounds (namely caffeine, metoprolol and midazolam) for CYP1A2, CYP2D6 and CYP3A4 during pregnancy within twofold of observed values. The changes during the third trimester were predicted to be a 100% increase, a 30% decrease and a 35% decrease in the exposure of caffeine, metoprolol and midazolam, respectively, compared with the nonpregnant women.

CONCLUSIONS

In the absence of clinical data, the *in silico* prediction of PK behaviour during pregnancy can provide a valuable aid to dose adjustment in pregnant women. The performance of the model for drugs metabolized by a single enzyme to different degrees (high and low extraction) and for drugs that are eliminated by several different routes warrants further study.

Introduction

Pregnancy is associated with an array of 'normal' profound physiological, anatomical and biochemical changes in the maternal body to accommodate the formed fetoplacental unit. These changes affect almost all maternal organs and are likely to alter the pharmacokinetics (PK) and pharmacodynamics of drugs administered during pregnancy significantly [1, 2]. A comprehensive meta-analysis of major changes that are known for their effects on drug kinetics has been carried out recently by our group [3]. Physiologically, all the temporal adaptations that affect the maternal body start simultaneously with conception, albeit with different magnitudes and trends [3]. Such a complex dynamic system challenges practical PK predictions during pregnancy (because all PK variables change with time) unless the study is carried out in a relatively short time span compared with the duration of pregnancy.

For clinical and ethical reasons, pregnant women are actively excluded from drug studies [4–6]. Frequently, if pregnancy occurs during a clinical study, the subject is dropped from the study. The majority of antenatally prescribed drugs are 'off-label' or unlicensed for pregnant women [7, 8], owing to a scarcity of studies in this group of patients. Drug dosing during pregnancy is usually scaled up from men or nonpregnant women, ignoring the impact of physiological and biochemical changes that occur during pregnancy. Such simple scaling may lead to over- or underdosing [9, 10]. For drugs with narrow safety windows, dose adjustment at various stages of pregnancy is necessary to avoid adverse drug effects [10–12]. Although overdosing has been a bigger concern, the failure to achieve the desired therapeutic effectiveness by underdosing can also put the pregnant mother and/or her fetus at risk.

The US Food and Drug Administration (FDA) [13, 14] and European Medicines Agency (EMA) [15] established a framework for the evaluation of the study designs, PK and monitoring of exposures to medicinal products during pregnancy. They have required that application holders make postmarketing commitments to conduct studies of the effects of products when used during pregnancy, especially for biopharmaceutical preparations that have a high likelihood of use in women of child-bearing potential. The EMA guidance reviews various study design options that may be used to monitor the safety of product exposures in pregnancy, while the FDA guidance document focuses on establishing prospective pregnancy exposure registries.

Currently, there is no widely accepted approach for appropriate optimization of the dose in pregnant women. The pregnancy physiologically based pharmacokinetic (p-PBPK) model that incorporates the pregnancy-induced changes in various anatomical, physiological and biological parameters is a feasible alternative to empirical dosage selection when there are no clinical studies to guide the requirements. Theoretically, a p-PBPK model will be useful not only in dosage adjustment for the pregnant popula-

tion, but also in developing drugs that are aimed only at pregnant women (e.g. tocolytic agents or drugs for pre-eclampsia). Nonetheless, these models require comprehensive information on the magnitude of anatomical, physiological and biochemical changes from nonpregnant status through to term and a reasonable knowledge of the drug disposition using *in vitro* information from a nonpregnant population.

A number of p-PBPK models have been reported for xenobiotic disposition in pregnant women. Recently, these models have been reviewed by our group [16]. Many of these were applications related to toxicokinetic and risk assessments in the mother and fetus [17–19]. Only two publications could be identified that focused on PK. One of these described the effect of pregnancy on the PK of midazolam disposition [20] and the other was related to gestagenic drugs [21]. This gap in the arena of PBPK modeling during pregnancy seems to be related to limitations of the data and the complexity of the required model. Recently, our group collected data for most of the 'drug-independent' (system) parameters required for p-PBPK models. These were subsequently analysed to investigate trends during pregnancy [3]. We have now incorporated these trends within the Simcyp® Simulator to create a p-PBPK model that is capable of modeling altered maternal PK for the whole duration of the pregnancy. Three model compounds that undergo hepatic metabolism by different cytochrome P450 enzymes, namely caffeine (a CYP1A2 substrate), metoprolol (a CYP 2D6 substrate) and midazolam (a CYP3A4 substrate), were used as case examples, and simulations were compared against clinical observations.

Methods

Model development

Physiological parameters required for p-PBPK model specifications and their changes during pregnancy were taken from a recently published meta-analysis of physiological, biological and anatomical measurements [3]. Formulas describing parameter values with gestational status were incorporated into the p-PBPK model. The p-PBPK model was implemented in Matlab using Simulink R2010a (The Mathworks, Inc., Natick, MA, USA). Unlike the Simcyp® Simulator, no variability terms were added to the model. Thus, the simulations represented only the average plasma drug levels.

Model structure A basic perfusion-limited version of a PBPK model consisting of 13 compartments representing various tissues was adopted from the Simcyp full-PBPK model [22]. The PBPK model was extended by the addition of an extra mechanistic compartment for intrauterine contents, renamed the fetoplacental unit, which is another perfusion-limited compartment running in parallel with the other maternal compartments that exist between the

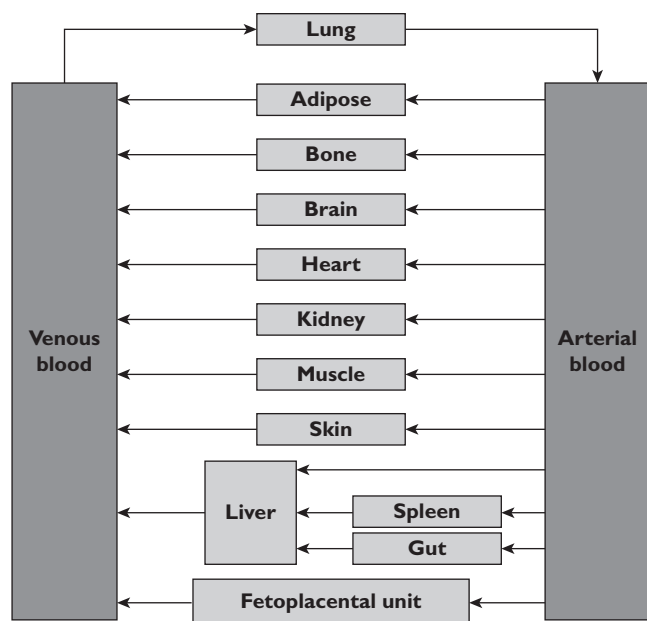


Figure 1
Schematic description of pregnancy physiologically based pharmacokinetic (p-PBPK) model

arterial and venous blood compartments (Figure 1). For model simplicity, the fetoplacental unit compartment combined all the following components: the fetus, placenta, amniotic fluid, membranes and umbilical cord. Additionally, the uterus and the mammary glands were grouped into the maternal muscle compartment. This was justified, because the focus of study was to evaluate the PK in the mother rather than assessing the exposure of the fetus to the drug.

Basal values for physiological parameters Mean values of all PBPK parameters that are implemented in Simcyp® Simulator version 11 for nonpregnant healthy Caucasian women aged between 20 and 40 years (i.e. child-bearing age) were used as basal values for the prepregnancy status (Table 1).

The basal values for the volume of the fetoplacental unit compartment and its blood flow rate were set to zero. As the uterus and mammary glands were grouped into the maternal muscle compartment, the tissue volume and blood flow rate of the muscle compartment were used to balance the allocation of total bodyweight and cardiac output in the model during pregnancy.

Time dependence of various parameters The longitudinal change in the fetoplacental unit volume during pregnancy was described according to the Gompertz equation as follows:

$$V_{\text{preg}} = a \cdot e^{(b/c)(1-e^{-c \cdot GA})} \quad (1)$$

Table 1

Basal value allocated for prepregnancy status tissue volumes and blood flow rates in the pregnancy physiologically based pharmacokinetic (p-PBPK) model

Tissue compartment	Volume, V (% of total body volume)	Blood flow rate, Q (% of cardiac output)
Adipose	0.385	0.085
Bone	0.025	0.05
Brain	0.016	0.12
Gut	0.015	0.17
Heart	0.003	0.05
Kidney	0.0037	0.17
Liver	0.02	0.28
Lung	0.004	1
Muscle	0.278	0.12
Skin	0.0298	0.03
Spleen	0.0018	0.05
Fetoplacental unit	0	0
Arterial blood	0.0205	1
Venous blood	0.0411	1

where *GA* denotes the gestational age in weeks and *a*, *b* and *c* are constants equal to 0.01, 0.37 and 0.052, respectively [3]. The polynomial equation was not superior to the Gompertz equation because it predicts a negative volume at an early time point in pregnancy (data not shown). The use of the Gompertz equation to describe fetal growth has been reported elsewhere [23].

Various other systemic parameters were described using polynomial formulae that implement gestational age-dependent changes [3]. On account of the basal values used in this study, these polynomial formulae are generalized as follows:

$$X = X_0 (a_0 + a_1 GA + a_2 GA^2 + a_3 GA^3) \quad (2)$$

where *X* denotes the physiological parameter as a function of the gestational age (*GA*, in weeks; $GA \geq 0$), X_0 is the basal value in the the prepregnant or nonpregnant state, and a_0 , a_1 , a_2 and a_3 are coefficients identified from meta-analysis of clinical and literature data.

This polynomial formula (Equation 2) was applied in the p-PBPK model to describe many parameters; a list of these parameters and their coefficients is given in Table 2. Furthermore, the total blood volume, which was calculated as plasma volume plus red blood cell volume, was further divided into the arterial and venous parts in the proportion of 1:2. Based on haematocrit and serum albumin, the fraction of drug unbound in plasma (f_u) and blood : plasma concentration ratio (*B : P*) were determined.

Gestational time scale In order to consider the time-varying coefficients included in the model, the PK time *t* (in

Table 2

Gestational age-dependent parameters used in the developed pregnancy model

Parameter, symbol (unit)	X_0	a_0	a_1	a_2	a_3
Cardiac output, Q_c (l h ⁻¹)	300	1	0.01965	-0.000292	0
Total bodyweight, BW (kg)	60	1	0.00560	0.000054	0
Total fat mass, V_{adip} (kg)	25.89	1	0.00035	0.000152	0
Plasma volume, V_{plas} (l)	2.71	1	-0.00892	0.00168	-0.000028
Red blood cell volume, V_{rbc} (l)	1.45	1	0.00658	0	0
Hematocrit, Hct (%)	38.3	1	-0.0014	-0.000054	0
Serum albumin, (g l ⁻¹)	44.9	1	-0.00358	-0.000085	0
Skin blood flow rate, Q_{skin} (l h ⁻¹)	14.975	1	0.02882	0	0
Adipose blood flow rate, Q_{adip} (l h ⁻¹)	25.458	1	0.01542	-0.000220	0
Renal blood flow rate, Q_{kidn} (l h ⁻¹)	50.915	1	0.05022	-0.00125	0
Fetoplacental unit blood flow rate, Q_{preg} (l h ⁻¹)	1	0	-0.4051	0.1188	-0.0019
CYP1A2 activity, α_{1A2}	1	1	-0.03581	0.00050	0
CYP2D6 activity, α_{2D6}	1	1	0.02270	-0.00035	0
CYP3A4 activity, α_{3A4}	1	1	0.02983	-0.00074	0

hours) was defined as a secondary time scale, which lay within the longer gestational time scale, at the point of administration of the drug GA_0 (in weeks). Therefore, gestational age GA at time t is given as follows:

$$GA = GA_0 + \frac{t}{24 \times 7} \quad (3)$$

where t denotes the PK simulation time, which is equal to the elapsed time (in hours) after the xenobiotic exposure, GA_0 is the gestational age in weeks when the pregnant woman is exposed to the xenobiotics, and 24 and 7 are constants used for conversion. At the time of conception $GA = 0$, whereas for nonpregnancy status $GA < 0$.

Governing equations As is common with all PBPK models, the governing equations in the p-PBPK model describing the concentration dynamics of xenobiotics were based on mass conservation law. The concentration dynamics in the fetoplacental unit compartment are described as follows:

$$\frac{d(V_{preg}C_{preg})}{dt} = Q_{preg} \left(C_{ab} - \frac{C_{preg}}{\frac{K_{preg:P}}{B:P}} \right) \quad (4)$$

where V_{preg} is the volume of the fetoplacental unit, C_{preg} denotes the xenobiotic concentration in the fetoplacental unit, Q_{preg} is the blood flow rate into the fetoplacental unit, C_{ab} is the xenobiotic concentration in the arterial blood, $K_{preg:P}$ is the fetoplacental unit:plasma partition coefficient, and $B:P$ is the blood:plasma concentration ratio. All of the parameters V_{preg} , Q_{preg} , $K_{preg:P}$ and $B:P$ are dependent on gestational time. Given that the pharmacokinetic time scale (in hours) is shorter than the gestational length (in

weeks), the left-hand side of the previous equation can be approximated as $V_{preg} \frac{dC_{preg}}{dt}$, thus:

$$V_{preg} \frac{dC_{preg}}{dt} = Q_{preg} \left(C_{ab} - \frac{C_{preg}}{\frac{K_{preg:P}}{B:P}} \right) \quad (5)$$

Similar differential equations are available in the other 13 maternal compartments as shown in Figure 1. Therefore, the model consists of 14 nonlinear differential equations, which are characteristic of time-varying coefficients.

Model validation

Validation set The p-PBPK model predictions were compared with different sets of clinical observations available in the literature for caffeine, metoprolol and midazolam. Selection of data for these probes was based on the availability of the relevant data, i.e. PK results, estimates and concentration–time profiles, from pregnant and nonpregnant Caucasian women and the information regarding the gestational time-dependent changes of their metabolizing enzymes. Simulation-matched study designs were used based on information in the original studies.

1 Caffeine is a well-known CYP1A2 substrate. Its pharmacokinetics were studied in eight pregnant women at 36 ± 3 weeks of gestation, showing a threefold increase in the half-life and threefold decrease in the total clearance during pregnancy compared with results from 4 days postpartum in four women [24]. An increasing half-life of caffeine with progression of pregnancy has been demonstrated [25–27]. By 3–15 weeks postpartum, the mean value of caffeine half-life was within the prepregnancy range [26].

Table 3

Pharmacokinetic parameters of caffeine, midazolam and metoprolol

Parameter	Symbol (unit)	Caffeine	Metoprolol	Midazolam
Fraction available for absorption from dosage form	f_a	1	0.88	0.88
Gut availability	F_g	1	0.99	0.59
First-order absorption rate constant	k_a (1 h ⁻¹)	2.18	1.45	3.04
Fraction of drug unbound in plasma	f_u	0.681	0.879	0.032
Blood : plasma concentration ratio	$B : P$	0.98	1.127	0.664
Intrinsic hepatic clearance	$CL_{int,H}$ (l h ⁻¹)	8	195	1583
CYP1A2 contribution to hepatic clearance	A_{1A2}	1	0	0
CYP2D6 contribution to hepatic clearance	A_{2D6}	0	0.93	0
CYP3A4 contribution to hepatic clearance	A_{3A4}	0	0.07	1

2 Metoprolol, a CYP2D6 substrate, was studied in five pregnant women at an average of 37 weeks of gestation and repeated 3–6 months after parturition [28]. It was concluded that the greater metoprolol clearance during pregnancy resulted from increased hepatic metabolism of the drug.

3 Midazolam undergoes extensive metabolism by hepatic CYP3A4 enzymes. Its PK evaluation during pregnancy was studied in 13 women (2 mg orally) at 28–32 weeks of gestation and at 6–10 weeks postpartum [29]. During pregnancy, the area under the concentration–time curve (AUC) to infinity and the maximal concentration (C_{max}) were decreased by 46 and 28%, respectively, compared with postpartum results. No significant changes were observed for the half-life and the time to C_{max} in the two cases.

Drug-dependent values Drug-dependent parameter values for these compounds were taken from default Simcyp library files (summarized in Table 3). The definition and symbol for different parameters are provided in Table 3 too.

For the sake of simplicity, all of f_a , F_g and k_a have been assumed to be constant in the following simulations, while f_u , $B:P$ and $CL_{int,H}$ depend on the pregnancy-induced changes of haematocrit, plasma albumin and hepatic enzyme activities during pregnancy.

Based on the activities of hepatic CYP1A2, CYP2D6 and CYP3A4, the change of $CL_{int,H}$ was defined as follows:

$$CL_{int,H} = CL_{int,H0} (\alpha_{1A2} A_{1A2} + \alpha_{2D6} A_{2D6} + \alpha_{3A4} A_{3A4}) \quad (6)$$

where $CL_{int,H0}$ is the basal hepatic intrinsic clearance in the nonpregnant women, expressed in the model as a ratio of basal value. A_{1A2} , A_{2D6} and A_{3A4} denote the contribution fraction of various metabolic pathways at gestational age GA to $CL_{int,H0}$ in the metabolism of xenobiotic; therefore, $A_{1A2} + A_{2D6} + A_{3A4} = 1$ (Table 3). The enzyme activity is represented as α and is dependent on gestational age (Tables 2 and 3).

Table 4

Tissue:plasma partition coefficients of caffeine, midazolam and metoprolol

Tissue	Caffeine	Metoprolol	Midazolam
Adipose	0.191	0.407	9.334
Bone	0.374	1.161	7.712
Brain	0.606	1.775	7.04
Gut	0.578	2.524	5.62
Heart	0.583	2.643	1.805
Kidney	0.597	2.475	2.725
Liver	0.57	3.379	3.4
Lung	0.62	1.553	0.728
Muscle	0.622	2.683	2.842
Skin	0.6	1.36	3.436
Spleen	0.591	2.732	2.757
Fetoplacental unit*	0.606	1.775	7.04

*Assumed to be the same as that of brain.

The values of $CL_{int,H0}$ and the total contribution of different metabolic pathways to the total $CL_{int,H0}$ were taken from Simcyp compound profiles, which are extrapolated from *in vitro* data for the respective relevant metabolic enzymes for these drugs [30].

In addition, Table 4 summarizes the tissue:plasma partition coefficients for the three aforementioned xenobiotics estimated using Rodgers and Rowland equations [31, 32]. Given that the data on the fetoplacental tissue:plasma partition coefficient ($K_{preg,P}$) are not available from the literature, the same values as brain:plasma partition coefficients are assumed for the compartment of the fetoplacental unit for simulation. This is mainly based on their similar characteristics, such as high blood perfusion and existence of blood–tissue barriers. All of these tissue:plasma partition coefficients are assumed to be constant during pregnancy.

Results

Caffeine pharmacokinetics profile

Simulated plasma caffeine concentrations for pregnant and nonpregnant women showed good agreement with the

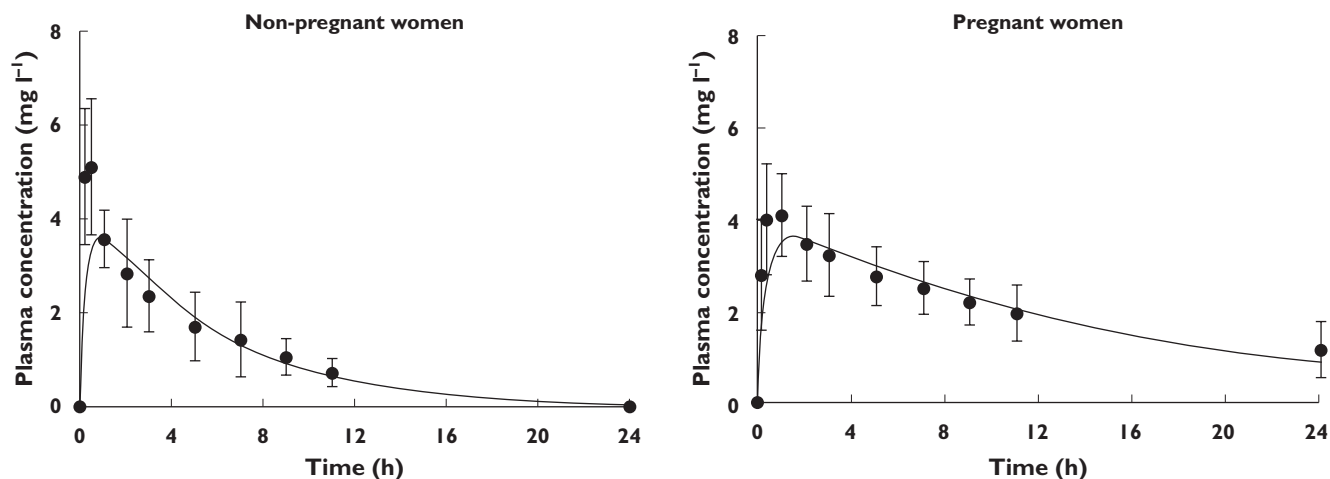


Figure 2

Simulated mean plasma total caffeine concentrations in nonpregnant ($n = 4$) and pregnant women ($n = 8$). —, predictions; •, Brazier *et al.* [24]

clinical observations (Figure 2). These plots demonstrated that the model replicated the observed higher plasma exposure obtained in pregnant women than in the nonpregnant women. A comparison of observed and predicted PK parameters for caffeine is given in Table 5.

Metoprolol pharmacokinetics profile

Simulated metoprolol concentration–time profiles were comparable to the observation obtained for metoprolol level in both pregnant and nonpregnant women (Figure 3). The simulated profiles for the intravenous dose indicated a lower AUC_{12h} for the pregnant ($143 \text{ ng ml}^{-1} \text{ h}^{-1}$) compared with the nonpregnant women ($164 \text{ ng ml}^{-1} \text{ h}^{-1}$). A similar trend for the AUC_{12h} was also found for the oral dose, $414 \text{ ng ml}^{-1} \text{ h}^{-1}$ for pregnant and $585 \text{ ng ml}^{-1} \text{ h}^{-1}$ for nonpregnant women.

Calculated pharmacokinetic parameters of metoprolol from simulated profiles are given in Table 5, in comparison with those obtained for clinical profiles.

Midazolam pharmacokinetics profile

Midazolam simulation results are shown in Figure 4. The model was able to describe the elimination profiles of midazolam and the overall lower exposure in pregnant compared with nonpregnant women; however, the distribution phase was underpredicted. The model prediction for the AUC_{6h} was $7.1 \mu\text{g l}^{-1} \text{ h}^{-1}$, which is lower than that in the control group ($10.8 \mu\text{g l}^{-1} \text{ h}^{-1}$). This corresponds to 35% difference between the two groups. Comparison of the PK parameters obtained for the simulated profiles with those from clinical study are given in Table 5.

Total vs. unbound pharmacokinetic profiles in pregnancy

As shown in Figures 5 and 6 and also in Table 5, there is a big difference between the total plasma concentration

and the unbound plasma concentration for midazolam. However, the AUC ratios and C_{max} ratios of all three drugs for pregnancy/nonpregnancy assessed using unbound concentrations showed only a slight difference relative to the corresponding values based on total concentrations, with the exception of midazolam (a highly bound drug). According to simulation results of various ‘what-if’ scenarios, different contributions of time-dependent changes in plasma protein binding and enzyme activity were observed. In the case of caffeine and metoprolol, a change in the enzyme activity during pregnancy had a big impact on AUC, but not on C_{max} . In the case of midazolam, the contribution of enzyme activity was similar to that of protein binding. As shown in Figure 6, the impact of a change in protein binding on C_{max} and AUC could be in the opposite direction from that for the enzyme activity (the caffeine case).

Discussion

The p-PBPK model developed here considers the gestational time-dependent parameters, which is a major advance on previous models according to a recent review [16] that enables the simulation of kinetics at any stage of pregnancy. This is, to our knowledge, the first p-PBPK model incorporating time-dependent activity of cytochrome P450 enzymes that is parameterized dynamically throughout pregnancy (as opposed to the discrete option of pregnant vs. nonpregnant status).

Previously developed p-PBPK models suffer from uncertainty in many of their parameters that account for the gestational time-dependent parameters. Therefore, various growth equations were used to generate the unavailable data [20, 33–37]. The assumption used for many values, such as constant metabolism throughout

Table 5
Comparison between observed and simulated pharmacokinetic parameters

Drug	PK parameter	Pregnant women			Nonpregnant women			Fold change from control		
		Observed	Predicted	Ratio (predicted/observed)	Observed	Predicted	Ratio (predicted/observed)	Observed	Predicted	Ratio
		Mean (SD)	Mean (unbound)		Mean (SD)	Mean (unbound)		Mean (total)	Mean (unbound)	
Caffeine 150 mg oral	<i>n</i>	8	–	–	4	–	–	–	–	–
	<i>C</i> _{max} (mg l ⁻¹)	4.58 (1.2)	3.26	2.67	6.02 (1.8)	3.59	2.44	↓0.76	↓0.91	↑1.09
	<i>T</i> _{max} (h)	1.5	1.5	1.5	0.75†	0.97	1	↑2.00	↑1.55	↑1.5
	AUC _{24h} (mg l ⁻¹ h ⁻¹)	52	47	36.41	2.6	24	16.36	↑2.00	↑1.96	↑2.23
	CL (l h ⁻¹)	2.86 (0.54)	3.19‡	–	7.17 (2.43)	6.25‡	–	↓0.40	↓0.51	–
Metoprolol 100 mg oral	<i>n</i>	5	–	–	5	–	–	–	–	–
	<i>C</i> _{max} (µg l ⁻¹)	60 (61)	126	120	180 (67)	201	186	↓0.33	↓1.60	↓1.55
	<i>T</i> _{max} (h)	1.7 (0.3)	0.5	0.4	1.1 (0.8)†	0.5	0.3	↑0.65	1.00	↓0.94
	AUC _{12h} (µg l ⁻¹ h ⁻¹)	219 (277)	414	391	835 (389)	585	516	↓3.81	↓1.41	↓1.32
	AUC _{24h} (µg l ⁻¹ h ⁻¹)*	188 (67)	143	135	310 (89)	164	144	↓1.65	↓1.15	↓1.07
CL (l h ⁻¹)	573.6 (162)	241.5‡	–	102.6 (23.4)	170.9‡	–	↑5.59	↑1.41	–	
Midazolam 2 mg oral	<i>n</i>	13	–	–	13	–	–	–	–	–
	<i>C</i> _{max} (µg l ⁻¹)	6.4 (2.6)	3.85	0.1	9.3 (2.0)	6.48	0.3	↓1.45	↓1.68	↓2.05
	<i>T</i> _{max} (h)	0.56 (0.15)	0.36	0.36	0.54 (0.14)†	0.39	0.38	↑0.96	↓1.08	↓1.05
	AUC _{6h} (µg l ⁻¹ h ⁻¹)	9.5 (4.3)	7.05	0.23	17.9 (6)	10.8	0.42	↓1.88	↓1.53	↓1.86
CL (l h ⁻¹)	252 (108)	284‡	–	120 (36)	185‡	–	↑2.10	↑1.54	–	

AUC, area under the concentration-time profile; CL, clearance; *C*_{max}, the maximum concentration; *n*, number of women; *T*_{max}, the time at which *C*_{max} occurs. *For 10 mg intravenous formulation. †Extracted from reported figures of caffeine [24], metoprolol [28] and midazolam [29]. ‡Calculated from dose/AUC_{24h} for caffeine, dose/AUC_{12h} for metoprolol and dose/AUC_{6h} for midazolam.

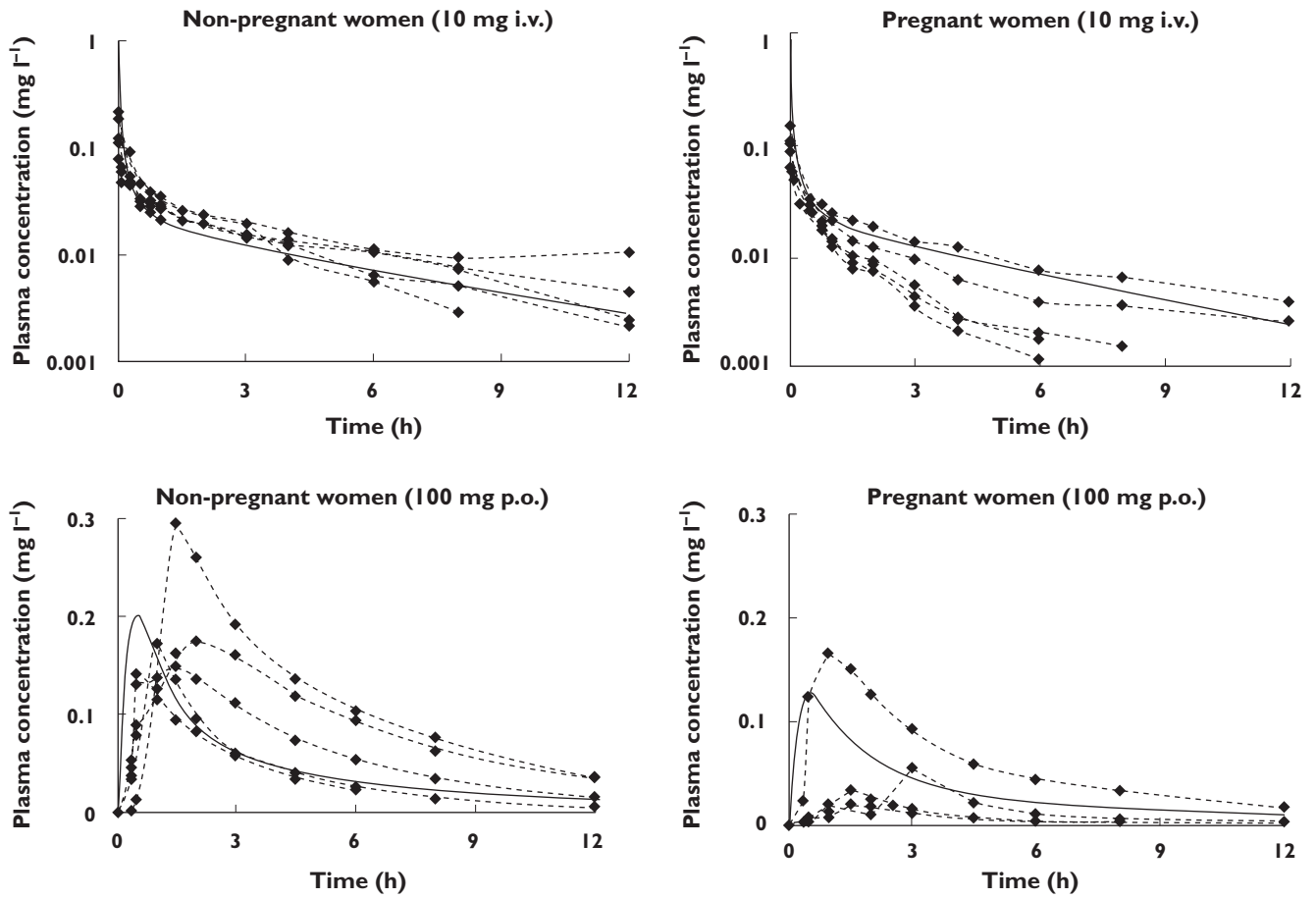


Figure 3

Simulated mean plasma total metoprolol concentrations in pregnant and nonpregnant women after administration of a 10 mg intravenous (upper two figures) or a 100 mg oral (lower two figures) single dose of metoprolol. —, predictions; -♦-, Hogstedt *et al.* [28] ($n = 5$)

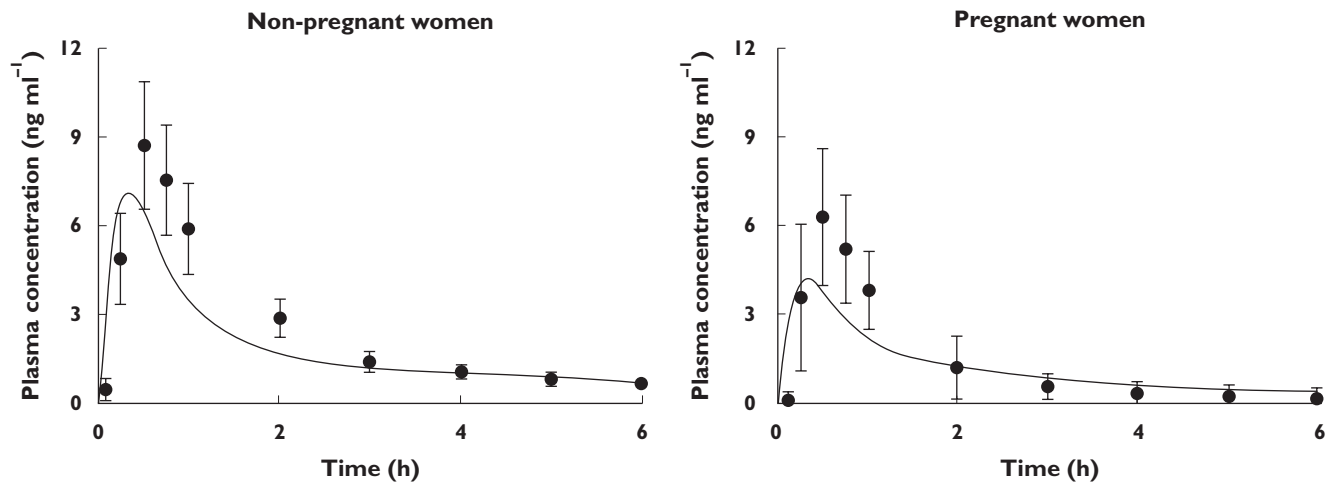
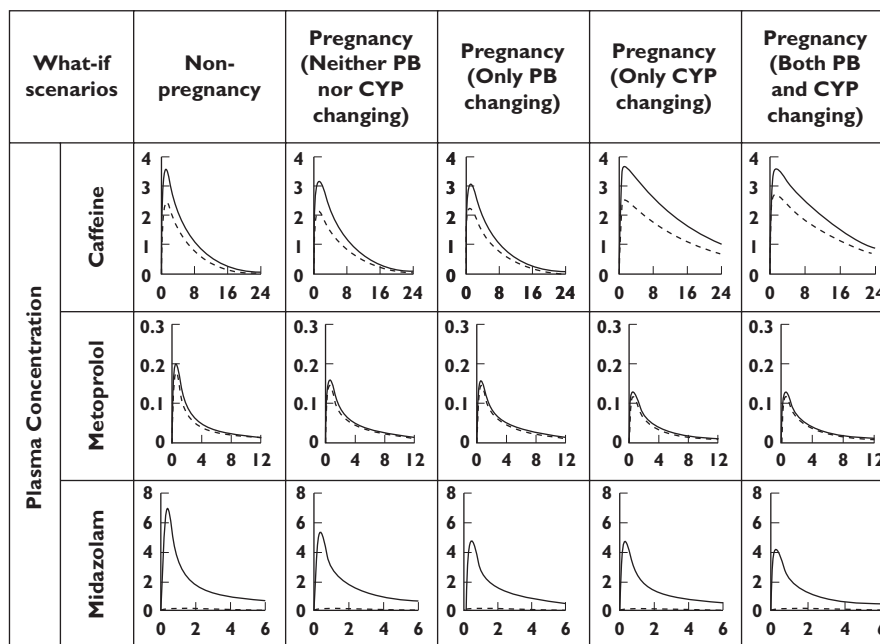


Figure 4

Simulated mean plasma total midazolam concentrations in pregnant and nonpregnant women. —, predictions; •, Hebert *et al.* [29] ($n = 13$)

**Figure 5**

Comparison of total plasma concentrations (continuous lines) to unbound plasma concentrations (dashed lines) in different 'what-if' scenarios accounting for changes of plasma protein binding (PB) and/or metabolism (CYP) during pregnancy. The horizontal axis is time (in hours). The vertical axis is plasma concentration (in mg l^{-1} for caffeine and metoprolol and in ng ml^{-1} for midazolam). All the scenarios are based on the same oral dose of 150 mg for caffeine, 100 mg for metoprolol and 2 mg for midazolam

pregnancy, limits their application to a specific compound at specific gestational times. Unlike these models, the proposed p-PBPK model in the present study was parameterized based on meta-analyses of combined physiological values (particularly those of enzyme activity for CYP1A2, CYP3A4 and CYP2D6) that vary throughout the gestational period [3]. These features enable the model to predict the pharmacokinetics of drugs eliminated via combination of the three enzymes studied and renal excretion at any time point during the pregnancy (personal correspondence, Alice Ke, University of Washington).

The structure of the current PBPK model is different from those of previous models, because the focus here was the maternal exposure. Andrew's model consists of 20 maternal compartments and 16 fetal compartments [20], while Luecke's model is comprised of 26 maternal compartments and 15 embryo/fetal compartments [33]. The latter was formulated as a set of 41 differential equations, while there are only 14 differential equations in the current model. The simplification in the current model reduces the uncertainty stemming from fetal physiological parameters; however, as a result of this simplification no concentration estimate in the fetal compartments can be made.

The model was validated using three different probes, namely caffeine, midazolam and metoprolol. Despite the limitations related to the small validation set, the focus on drugs that are predominantly metabolized by a single enzyme, and assumptions regarding absence of changes

in gut wall metabolism (see later discussion), it appears that the model could form the nucleus for further development and, moreover, it identifies the knowledge gaps to be addressed.

Irrespective of the small study size for caffeine (four nonpregnant and eight pregnant women), the model is able to describe the concentration profiles of caffeine in pregnant and nonpregnant women (Figure 2). The model predicted a twofold increase in caffeine exposure near term compared with that in nonpregnant women. The $\text{AUC}_{24\text{h}}$ increased from $24 \text{ mg l}^{-1} \text{ h}^{-1}$ in nonpregnant women to $50 \text{ mg l}^{-1} \text{ h}^{-1}$ near term. These values are consistent with the reported values for nonpregnant vs. pregnant women (26 vs. $52 \text{ mg l}^{-1} \text{ h}^{-1}$) [24]. This increase in AUC corresponds to a twofold decrease in total clearance [24, 25], most probably because of the decrease in CYP1A2 activity during pregnancy [38, 39]. In both study arms, the predictability of the constructed model to give similar PK parameters is given in Table 5.

The obtained lower $\text{AUC}_{12\text{h}}$ of metoprolol for pregnant women compared with the control group was in agreement with clinical studies showing higher clearance of metoprolol during pregnancy. During pregnancy, metoprolol clearance, $CL_{i,v}$, was increased from $39.0 \pm 10.7 \text{ l h}^{-1}$ in five nonpregnant women to $82.8 \pm 34.9 \text{ l h}^{-1}$ in five pregnant women at term [28]. This increase in clearance during pregnancy results from increased hepatic metabolism of metoprolol [28] due to increased CYP2D6 activity

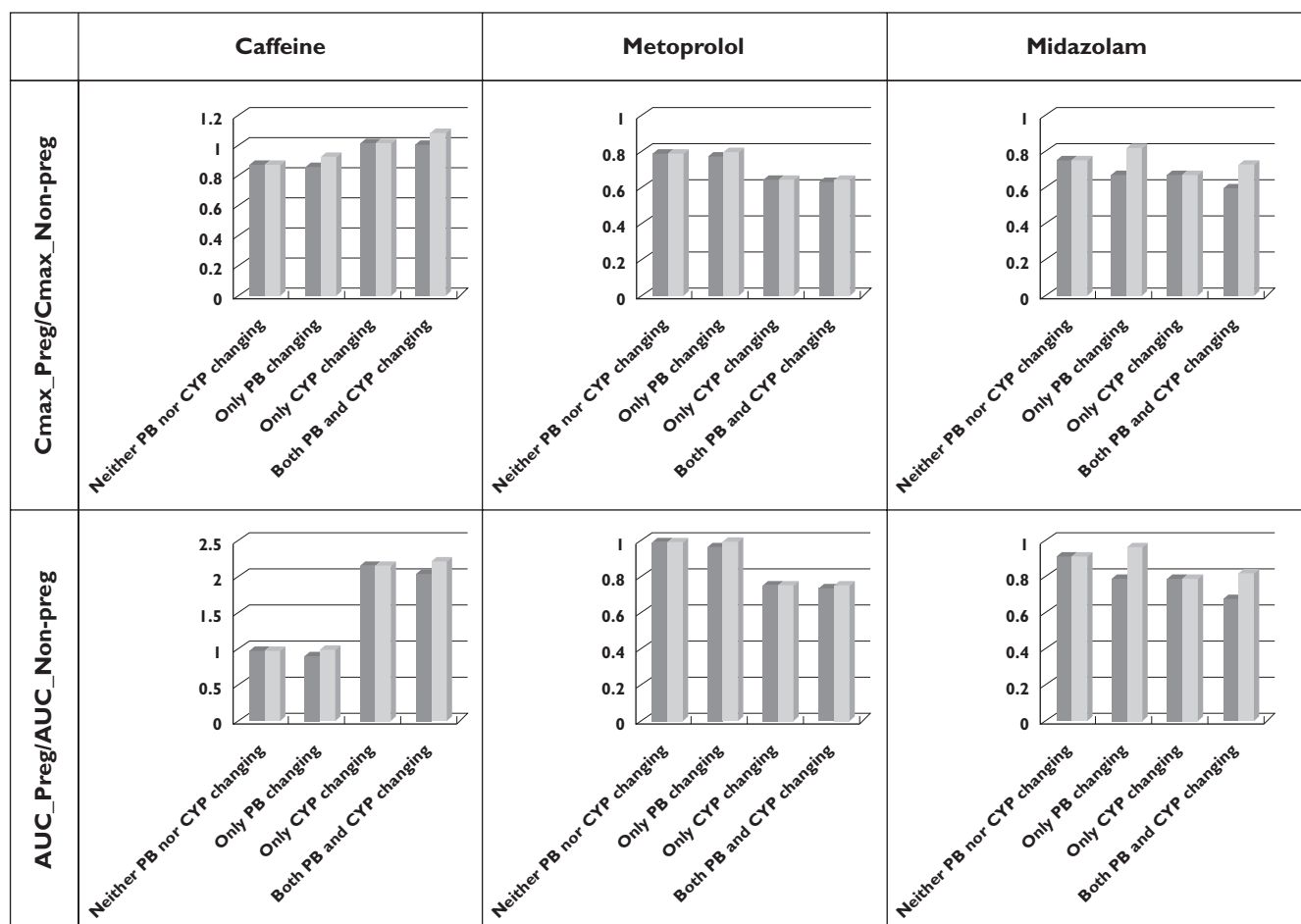


Figure 6

Contributions of changes to plasma protein binding and metabolism during pregnancy to variation of C_{max} and AUC during pregnancy. Dark grey columns are based on the total plasma concentration and pale grey columns are based on the unbound plasma concentration

during gestation [39, 40]. Data from those five women shows a 39% decrease in the AUC after intravenous administration and 47% after oral administration of metoprolol in pregnant women compared with nonpregnant women, while our simulation shows a decrease of 13% for intravenous administration and 35% for an oral dose (Table 5). This could be explained partly by the small size of the clinical study ($n = 5$) and the knowledge that the activity of CYP2D6 is highly variable among individuals and controlled by genetic factors [41, 42]. Information on individual CYP2D6 polymorphisms was neither reported in the original paper nor included in the model.

For midazolam, the p-PBPK model underpredicted the maximal concentrations in both groups of pregnant and nonpregnant women (Figure 4); however, the predicted values lie within the reported range. The model predicted a 35% difference in the AUC_{6h} between pregnant ($7.1 \mu\text{g l}^{-1} \text{h}^{-1}$) and nonpregnant values ($10.8 \mu\text{g l}^{-1} \text{h}^{-1}$). This difference is consistent with the clinical 46.9% difference calculated for the first 6 h after drug administration.

An underprediction of C_{max} occurred for caffeine. However, the C_{max} prediction could be improved by modifying the parameter setting of the first-order absorption, where three parameters are used, namely f_a (fraction available for absorption from dosage form), k_a (first-order absorption rate constant) and F_g (gut availability). As given in Table 3, for caffeine, $f_a = 1$, $F_g = 1$ and $k_a = 2.18 \text{ h}^{-1}$, while for midazolam, $f_a = 0.88$, $F_g = 0.59$ and $k_a = 3.04 \text{ h}^{-1}$. It is noteworthy that the current p-PBPK model and its simulation used only these default values provided by the Simcyp Simulator, which does not include any information from the three clinical pregnancy studies that were considered in this report.

As pharmacological effects are related to the unbound drug concentration, clinical consideration should be given to the possibility that pregnant women may need a change of dose if the exposure to unbound concentration changes (e.g. midazolam). Consideration of the total midazolam ratios for AUC and C_{max} suggests that a bigger dose increase is needed in pregnancy than necessary.

For drugs with a higher fraction unbound, such as caffeine ($f_u = 0.68$) and metoprolol ($f_u = 0.88$) in this study, both the total and the unbound ratios suggest that a similar magnitude of dose decrease and increase, respectively, is needed for these drugs. The utilization of a p-PBPK model to undertake 'what-if' simulations is useful to predict the changes in unbound drug concentration that are likely to occur during pregnancy and, subsequently, to provide information to guide dosing recommendations.

In general, model predictions for caffeine, midazolam and metoprolol demonstrated a good performance when compared against the clinical observations. The current model was developed to describe drug disposition in maternal organs; hence, no effort was made to model the fetoplacental unit in detail within the model structure. Nonetheless, the fetoplacental compartment was semi-mechanistic in that its volume and the blood flow from the maternal circulation were time dependent. The 'dummy' nature of the compartment and assumption on its homogeneity without subdivision into fetus, placenta, membranes, amniotic fluid and the umbilical cord, prevent any extrapolation to interpret the concentration within the fetoplacental unit. This could be considered in a future expansion of the model to consider both maternal and fetal exposure.

An assumption was made that the partition coefficient for this fetoplacental compartment is the same as that for the brain compartment. This assumption was made on the basis that the barriers to these tissues share some similar physiological and biochemical functions. Drug-metabolizing enzymes and transporters are expressed both in the placenta [43–45] and in the brain [46–49] and have a similar function in each organ; however, their significance is related to local kinetics rather than overall exposure in the body. Both of these barriers comprise anatomically tight subbarriers [46, 50, 51]. As the make-up of the placental barrier changes over the course of pregnancy [51], the assumed constant value of fetoplacental tissue:plasma partition coefficient may need mechanistic description, most probably, as a gestational time-varying parameter. From the sensitivity analysis of C_{max} , T_{max} and AUC in plasma and in the fetoplacental compartment with respect to the partition coefficient of the fetoplacental compartment (Appendix S1), it was observed that the system concentration is insensitive to this parameter, while the the fetoplacental compartment exposure to caffeine depends on this parameter.

Further sensitivity analysis on the impact of gestational age shows that both the plasma AUC and the pregnancy tissue AUC increased during pregnancy, with respect to the same oral caffeine consumption (Appendix S2). Both AUCs peaked at 35 weeks of gestation. In particular, the plasma AUC at this gestational age was twofold higher than pre-pregnancy (49.6 vs. 24.0 mg l⁻¹ h). Visibly,

the rate of increase in the pregnancy tissue AUC was larger in the first trimester compared with that in the second or third trimester.

Another short-coming of the current model was related to assumptions on constant metabolism in the gut wall throughout pregnancy. This may not have any impact on caffeine and metoprolol (which are metabolized by CYP1A2 and CYP2D6); however, sensitivity of the changes to gut wall metabolism requires some investigation for drugs such as midazolam (a CYP3A4 substrate) and other drugs with varying gut wall metabolism. As the value of F_g is dependent on the enterocytic blood flow of the gut wall, the permeability clearance of the gut wall, as well as the intrinsic metabolic clearance of the gut wall, PK prediction of the compounds with gut wall metabolism (midazolam is a representative compound) could be improved by considering the gut wall metabolism. The gut wall metabolism might be changing throughout pregnancy.

Finally, considerations for individual variability are not part of the model. Once the interindividual variability is added to model parameters, the model will be more useful in its ability to assist study design for clinical investigations of PK and dosage requirements during any specific gestational time period.

Conclusions

A simple, perfusion-limited compartmental PBPK model has been developed for Caucasian healthy pregnant women, which describes the kinetics in various stages of gestation. Time-dependent profiles for three major metabolizing enzymes, CYP1A2, CYP2D6 and CYP3A4, were included in the model and validated using three different sets of pharmacokinetic data in the pregnant women. Potential extensions to the model and further validation may include drugs with multiple elimination pathways, drugs with variable levels of gut wall metabolism and assessment of fetal exposure.

Competing Interests

There are no competing interests to declare.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1

Sensitivity analysis on $K_{\text{preg:P}}$ (pregnancy tissue:plasma partition coefficient)

Appendix S2

Sensitivity analysis on GA (gestational age)

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