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Prediction of pregnancy-induced changes in secretory and total renal clearance of drugs transported by organic anion transporters

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Prediction of renal clearance of OAT substrates during pregnancy

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d) Abbreviations:

OATs: organic anion transporters, OAT_j: OAT1, OAT2, or OAT3, F_{reabs}: fraction of drug reabsorbed, F: oral bioavailability, f_e: fraction of drug excreted unchanged in the urine, fu: fraction unbound of drug in plasma, $ft_{OAT\,j}$: fraction of drug transported by OAT_j, GFR: glomerular filtration rate, HCG: human chorionic gonadotropin, RAFs: relative activity factors; CL_{total}: plasma total clearance, $CL_{total,B}$: blood total clearance, CL_{renal}: plasma renal clearance, $CL_{renal,B}$: blood renal clearance, $CL_{non-renal,B}$: blood non-renal clearance, CL_{sec} : plasma renal secretory clearance, $CL_{sec,B}$: blood renal secretory clearance, $CL_{int,sec}$: intrinsic renal secretory clearance, $CL_{int,OAT\,j}$: individual OAT_j mediated clearance, $CL_{renal}^{pregnant}$: plasma renal clearance in pregnancy, $CL_{sec}^{pregnant}$: plasma renal secretory clearance in pregnancy, $CL_{total,B}^{pregnant}$: plasma total clearance in pregnancy, $CL_{renal,B}^{pregnant}$: blood renal clearance in pregnancy, $CL_{sec,B}^{pregnant}$: blood renal secretory clearance in pregnancy, $CL_{int,sec}^{pregnant}$: intrinsic renal secretory clearance in pregnancy, $CL_{int,OAT\,j}^{pregnant}$: individual OAT mediated intrinsic clearance in pregnancy, CL_{PD}: passive diffusion clearance, $CL_{int,PD}$: intrinsic passive diffusion clearance, PEPT: peptide transporter.

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Abstract (250/250)

Pregnancy can significantly change the pharmacokinetics of drugs, including those renally secreted by organic anion transporters (OATs). Quantifying these changes in pregnant women is logistically and ethically challenging. Hence, predicting the *in vivo* plasma renal secretory clearance ($\mathrm{CL_{sec}}$) and renal CL ($\mathrm{CL_{renal}}$) of OAT drugs in pregnancy is important to design correct dosing regimens of OAT drugs. Here, we first quantified the fold-change in renal OAT activity in pregnant vs. non-pregnant individual using available selective OAT probe drug $\mathrm{CL_{renal}}$ data (training dataset; OAT1: tenofovir, OAT2: acyclovir, OAT3: oseltamivir carboxylate). The fold-change in OAT1 activity during the 2nd and 3rd trimester was 2.9 and 1.0 compared to nonpregnant individual, respectively. OAT2 activity increased 3.1-fold during the 3rd trimester. OAT3 activity increased 2.2, 1.7 and 1.3-fold during the 1st, 2nd and 3rd trimester, respectively. Based on these data, we predicted the $\mathrm{CL_{sec}}$, $\mathrm{CL_{renal}}$ and $\mathrm{CL_{total}}$ of drugs in pregnancy, which are secreted by multiple OATs (verification dataset; amoxicillin, pravastatin, cefazolin and ketorolac, R-ketorolac, S-ketorolac). Then, the predicted CLs were compared with the observed values. The predicted/observed $\mathrm{CL_{sec}}$, $\mathrm{CL_{renal}}$ and $\mathrm{CL_{total}}$ of drugs in pregnancy of all verification drugs were within 0.80-1.25 fold except for $\mathrm{CL_{sec}}$ of amoxicillin in the 3rd trimester (0.76-fold) and cefazolin in the 2nd trimester (1.27-fold). Overall, we successfully predicted the $\mathrm{CL_{sec}}$, $\mathrm{CL_{renal}}$ and $\mathrm{CL_{total}}$ of drugs in pregnancy that are renally secreted by multiple OATs. This approach could be used in the future to adjust dosing regimens of renally secreted OAT drugs which are administered to pregnant women.

Keywords:

pregnancy, OAT transporters, renal secretory clearance prediction, *in vivo* to *in vivo* extrapolation

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Significance Statement (61 words, 3 sentences):

To our knowledge, this is the first report to successfully predict renal secretory clearance and renal clearance of multiple OAT-substrate drugs during pregnancy. The data presented here could be used in the future to adjust dosing regimens of renally secreted OAT drugs in pregnancy. In addition, the mechanistic approach used here could be extended to drugs transported by other renal transporters.

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Introduction

Pregnant women commonly take drugs (medication) throughout their pregnancy especially when afflicted with chronic diseases such as diabetes, hypertension or infectious diseases (e.g. human immunodeficiency virus). About 97% take at least one drug, and about 31% take at least five drugs during pregnancy (Mitchell *et al.*, 2011; Haas *et al.*, 2018). Due to lack of clinical trials of drug candidates in pregnancy, a large proportion of approved drugs are prescribed off-label to pregnant women (Laroche *et al.*, 2020). However, because pharmacokinetics of many drugs are modulated by pregnancy, such use may result in suboptimal dosing of pregnant women (Westin *et al.*, 2018). To design optimal drug dosing regimens for pregnant women, it is important to quantify the pregnancy-induced changes in pharmacokinetics of drugs. However, such studies are logistically and ethically challenging. Therefore, an alternative is to predict these changes by first characterizing, using probe drugs, the magnitude of pregnancy-induced changes for a given drug clearance pathway. We have previously used this approach to predict the pregnancy-induced changes in pharmacokinetics of drugs that are metabolized by cytochrome P450 enzymes (Ke *et al.*, 2012, 2013; Ke, Nallani, *et al.*, 2014). Here we extended this approach to drugs that are renally cleared via organic anion transporters (OAT) using published data of renal secretory clearance (CL) of probe drugs that are selectively transported by a single OAT.

In clinical practice, 32% of the top 200 prescribed drugs are renally eliminated (i.e. ≥25% of the absorbed dose is excreted unchanged in urine) (Morrissey *et al.*, 2013). Many of these drugs taken by pregnant women are secreted by single or multiple OAT (OAT1-3) such as antibiotics (e.g. amoxicillin, ampicillin) and antivirals (e.g. tenofovir, adefovir) (Mitchell *et al.*, 2011; Leong *et al.*, 2019). We have previously shown that P-gp- and/or OATP4C1-mediated secretory CL of

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digoxin is significantly increased during pregnancy (Hebert *et al.*, 2008; Ke, Rostami-Hodjegan, *et al.*, 2014). Likewise, the clearance of OAT-secreted drugs is also increased during pregnancy (e.g. amoxicillin and tenofovir (Andrew *et al.*, 2007; Best *et al.*, 2015)), suggesting that OAT activity is increased during pregnancy. To predict the magnitude of changes in OAT-mediated renal CL of drugs in pregnancy, we first quantified the magnitude of pregnancy-induced change in the activity of individual OATs (OAT1, 2, 3). This we did based on previously published renal secretory CL ($\mathrm{CL_{sec}}$) and total renal CL ($\mathrm{CL_{renal}}$) of OAT probe substrate drugs (training dataset; OAT1: tenofovir, OAT2: acyclovir, OAT3: oseltamivir carboxylate) that are known to be predominately renally cleared (> 75%) by a single OAT. Then, these pregnancy-induced fold-changes in individual OAT activity were used to predict the pregnancy-induced changes in renal secretory CL ($\mathrm{CL_{sec}^{pregnant}}$), renal CL ($\mathrm{CL_{renal}^{pregnant}}$) and total CL ($\mathrm{CL_{total}^{pregnant}}$) of drugs transported by a single or multiple OATs (verification dataset; amoxicillin, pravastatin, cefazolin and ketorolac, R-ketorolac, S-ketorolac). To do so, we estimated the fraction of drug transported (f_t) by the individual OAT (1, 2 and/or 3) based on their *in vitro* transporter-mediated and passive clearance data in OAT- or mock-transfected cell lines. Predictions by any approach must be verified before it can be applied with confidence to other drugs. Therefore, to verify the above predictions, we compared the predicted and the observed $\mathrm{CL_{sec}^{pregnant}}$, $\mathrm{CL_{renal}^{pregnant}}$ and $\mathrm{CL_{total}^{pregnant}}$ values of these drugs. We deemed our predictions successful if they fell within 80% to 125% of the observed values.

Methods

PK data collection and estimation of $\mathbf{CL_{int,sec}}$ of drugs

Briefly, renal total clearance ($\mathrm{CL_{renal,B}}$) of training drugs (tenofovir, acyclovir, oseltamivir carboxylate) and verification drugs (amoxicillin, cefazolin, pravastatin and ketorolac, R-

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ketorolac, S- ketorolac) in non-pregnant and pregnant women were obtained from the literature or estimated from the reported data. These data were available mostly for the 2nd and 3rd trimester. Except for acyclovir, where the PK parameters were from two different cohort of women (pregnant and non-pregnant), for the remaining drugs, the pharmacokinetic parameters were from the same cohort of women studied, antenatal (oseltamivir carboxylate and ketorolac) and postpartum (≥ 6 weeks). We assumed pharmacokinetic parameters obtained from postpartum women had returned to levels of non-pregnant women prior to pregnancy. Observed intrinsic secretory CL ($\mathrm{CL_{int,sec}}$), blood renal secretory CL ($\mathrm{CL_{sec,B}}$) and blood renal total CL ($\mathrm{CL_{renal,B}}$) of tenofovir, oseltamivir carboxylate, cefazolin, and ketorolac were calculated from their reported $\mathrm{CL/F}$ (po) or CL (IV). Observed CL of acyclovir was estimated by digitizing the reported concentration-time profile using WebPlotDigitizer (https://apps.automeris.io/wpd/). Observed $\mathrm{CL_{int,sec}}$ and $\mathrm{CL_{sec,B}}$ of amoxicillin and pravastatin were calculated from the reported $\mathrm{CL_{renal,B}}$. Details of how these parameters were estimated are provided below (Fig. 1. Step 1-3).

First, $\mathrm{CL_{renal,B}}$ in both non-pregnant and pregnant women were estimated (if not already stated in the publications) using equations (1) - (4) (Cho et al., 2019).

$$\mathrm{CL_{total,B}} = \frac{\mathrm{Dose} \times \mathrm{F}}{\mathrm{AUC_{inf}} \times \mathrm{BP}} \quad (1)$$

$$\mathrm{CL_{renal,B}^{non-pregnant}} = \mathrm{CL_{total,B}^{non-pregnant}} \times \mathrm{f_e} \quad (2)$$

$$\mathrm{CL_{non-renal,B}^{non-pregnant}} = \mathrm{CL_{total,B}^{non-pregnant}} \times (1 - \mathrm{f_e}) \quad (3)$$

$$\mathrm{CL_{renal,B}^{pregnant}} = \mathrm{CL_{total,B}^{pregnant}} - \mathrm{CL_{non-renal,B}^{non-pregnant}} \quad (4)$$

where $\mathrm{CL_{total,B}}$ was the sum of the $\mathrm{CL_{renal,B}}$ and non-renal clearance ($\mathrm{CL_{non-renal,B}}$). $\mathrm{AUC_{inf}}$ was the area under the plasma concentration-time profile from zero to infinity. Oral bioavailability (F) and fraction excreted in the urine as unchanged drug ($\mathrm{f_e}$) in non-pregnant women are provided

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in Table 1. F, f_e and $CL_{non-renal}$ of tenofovir, acyclovir, oseltamivir carboxylate, cefazolin and ketorolac were assumed to be unchanged by pregnancy. Blood to plasma ratio (BP) in nonpregnant or pregnant women was estimated using previous published methods (Uchimura *et al.*, 2010; Zhang *et al.*, 2017). Clearances were normalized by the non-pregnant women's body weight (i.e. postpartum weight for those women who were also studied antenatal) to take into account the effect of body weight on CL.

Then, the observed $CL_{sec,B}$ and $CL_{int,sec}$ in non-pregnant and pregnant women was calculated from $CL_{renal,B}$ using the well-stirred model (equations. 5-6) (Ladumor *et al.*, 2019).

$$CL_{sec,B} = \frac{CL_{renal,B}}{1 - F_{reabs}} - fu_B * GFR_{inulin} \quad (5)$$

$$CL_{int,sec} = \frac{Q_{renal} * CL_{sec,B}}{fu_B * (Q_{renal} - CL_{sec,B})} \quad (6)$$

where fraction unbound in blood (fu_B) was the ratio of fraction unbound in plasma (fu) and BP in nonpregnant or pregnant women estimated using previous published methods (Dallmann, Ince, Solodenko, *et al.*, 2017; Zhang *et al.*, 2017). Renal blood flow (Q_{renal}) were determined from gestational age dependent renal plasma flow and hematocrit (Hct, %) (Odutayo and Hladunewich, 2012; Dallmann, Ince, Meyer, *et al.*, 2017). Glomerular filtration rate (GFR_{inulin}) was based on inulin blood clearance in different trimesters (Koetje *et al.*, 2011; Odutayo and Hladunewich, 2012). As justified by others, F_{reabs} of the drugs was assumed to be negligible (Mathialagan *et al.*, 2017).

In addition, gestational stage in our study was defined per U.S. Department of Health and Human Services (HHS) recommendations: 1-12 weeks as the 1st trimester, 13-28 weeks as the 2nd trimester, and 29-40 weeks as the 3rd trimester. Except for oseltamivir carboxylate, drug CL

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data for the remaining probe drugs were not available for the 1st trimester. Likewise, CL data for acyclovir and ketorolac in the 2nd trimester and cefazolin in the 3rd trimester were not available.

Determination of modulation of OAT activity during pregnancy (Steps 4-5, Fig. 1)

As reported in our previous study (Kumar *et al.*, 2021), the *in vivo* passive diffusion clearance ($\mathrm{CL}_{\mathrm{int,PD}}$) of the drugs was estimated (Equation 7) from previously reported *in vitro* $\mathrm{CL}_{\mathrm{int,PD},in\ vitro}$ in transfected HEK293 cells (Mathialagan *et al.*, 2017)

$$\mathrm{CL}_{\mathrm{int,PD}}(\mathrm{mL/min/kg}) = \mathrm{CL}_{\mathrm{int,PD},in\ vitro} * \mathrm{MPPGK} * \mathrm{Kideny\ weight} \quad (7)$$

where MPPGK (i.e. milligram of total proteins per gram of kidney) is 15 mg/g and kidney weight is 4.3 g/kg body weight (Mathialagan *et al.*, 2017).

Then, the fold-change in OAT activity during pregnancy was calculated by equation 8.

$$\text{Fold change in OATj activity} = \frac{\mathrm{CL}_{\mathrm{int,OATj}}^{\mathrm{pregnant}}}{\mathrm{CL}_{\mathrm{int,OATj}}^{\mathrm{non-pregnant}}} = \frac{\mathrm{CL}_{\mathrm{int,sec}}^{\mathrm{pregnant}} - \mathrm{CL}_{\mathrm{int,PD}}}{\mathrm{CL}_{\mathrm{int,sec}}^{\mathrm{non-pregnant}} - \mathrm{CL}_{\mathrm{int,PD}}} \quad (8)$$

where $\mathrm{OAT_j}$ indicates OAT1, OAT2 or OAT3 (training dataset; OAT1: tenofovir, OAT2: acyclovir, OAT3: oseltamivir carboxylate; these drugs were assumed to be solely secreted by the listed OAT). $\mathrm{CL_{PD}}$ was assumed to be unchanged by pregnancy.

Determination of fraction of the verification drug transported by $\mathrm{OAT_j}$

Fraction transported by $\mathrm{OAT_j}$ ($\mathrm{ft_{OAT\,j}}$) of the verification drugs (amoxicillin, pravastatin, cefazolin and ketorolac, R-ketorolac, S-ketorolac) was calculated by Eq. 9.

$$\mathrm{ft_{OAT\,j}} = \frac{\mathrm{RAF_{OAT\,j}} * \mathrm{CL_{int,OAT\,j}}}{\sum_{j=1}^{3}(\mathrm{RAF_{OAT\,j}} * \mathrm{CL_{int,OAT\,j}})} \quad (9)$$

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where *in vitro* $\mathrm{CL_{int,OAT\,j}}$ and relative activity factor ($\mathrm{RAF_{OAT\,j}}$, 0.64, 7.3, and 4.1 for OAT1, OAT2, and OAT3, respectively) were obtained from a previous publication (Mathialagan *et al.*, 2017). Then, $\mathrm{ft_{OAT\,j}}$ was used to calculate the individual OAT-mediated $\mathrm{CL_{int,OAT_j}^{non-pregnant}}$ of verification drugs in non-pregnant individuals by equation 10.

$$\mathrm{CL_{int,OAT_j}^{non-pregnant}} = \mathrm{ft_{OAT\,j}} * (\mathrm{CL_{int,sec}^{non-pregnant}} - \mathrm{CL_{int,PD}}) \quad (10)$$

Prediction of $\mathbf{CL_{sec}}$, $\mathbf{CL_{renal}}$ and $\mathbf{CL_{total}}$ of the verification drugs in pregnancy

Based on the above data, $\mathrm{CL_{int,OAT_j}^{pregnant}}$ of the verification drugs was calculated by multiplying fold-change in activity of each OAT at different gestational ages with $\mathrm{CL_{int,OAT_j}^{non-pregnant}}$ in non-pregnant individuals (equation 11). $\mathrm{CL_{int,sec}^{pregnant}}$ was calculated from the total of individual $\mathrm{CL_{int,OAT_j}^{pregnant}}$ and the predicted *in vivo* $\mathrm{CL_{int,PD}}$ (equation 12). Further, $\mathrm{CL_{sec,B}^{pregnant}}$ was estimated using well-stirred model based on $\mathrm{Q_{renal}}$ and $\mathrm{fu_B}$ during pregnancy (equation 13). Finally, $\mathrm{CL_{renal,B}^{pregnant}}$ was determined from $\mathrm{CL_{sec,B}^{pregnant}}$, $\mathrm{fu_B}$ and $\mathrm{GFR_{inulin}}$ during pregnancy (equation 14). $\mathrm{F_{reabs}}$ was assumed to be negligible (Mathialagan *et al.*, 2017). Total clearance (based on blood concentrations) in pregnancy ($\mathrm{CL_{total,B}^{pregnant}}$) was predicted using equation 15, where non-renal CL was assumed to be unaffected by pregnancy and calculated using equation 16.

$$\mathrm{CL_{int,OAT\,j}^{pregnant}} = \text{Fold change in OATj activity} * \mathrm{CL_{int,OAT_j}^{non-pregnant}} \quad (11)$$

$$\mathrm{CL_{int,sec}^{pregnant}} = \sum_{3}^{1} \mathrm{RAF_{OAT\,j}} * \mathrm{CL_{int,OAT\,j}^{pregnant}} + \mathrm{CL_{int,PD}} \quad (12)$$

$$\mathrm{CL_{sec,B}^{pregnant}} = \frac{\mathrm{Q_{renal}^{pregnant}} * \mathrm{fu_B^{pregnant}} * \mathrm{CL_{int,sec}^{pregnant}}}{\mathrm{Q_{renal}^{pregnant}} + \mathrm{fu_B^{pregnant}} * \mathrm{CL_{int,sec}^{pregnant}}} \quad (13)$$

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$$\mathrm{CL}_{\mathrm{renal,B}}^{\mathrm{pregnant}} = \left(\mathrm{CL}_{\mathrm{sec,B}}^{\mathrm{pregnant}} + \mathrm{fu}_{\mathrm{B}}^{\mathrm{pregnant}} * \mathrm{GFR}_{\mathrm{inulin}}^{\mathrm{pregnant}}\right) * (1 - \mathrm{F}_{\mathrm{reabs}}) \quad (14)$$

$$\mathrm{CL}_{\mathrm{total,B}}^{\mathrm{pregnant}} = \mathrm{CL}_{\mathrm{renal,B}}^{\mathrm{pregnant}} + \mathrm{CL}_{\mathrm{non-renal,B}}^{\mathrm{non-pregnant}} \quad (15)$$

$$\mathrm{CL}_{\mathrm{non-renal,B}}^{\mathrm{non-pregnant}} = \mathrm{CL}_{\mathrm{renal,B}}^{\mathrm{non-pregnant}} * \left(\frac{1}{\mathrm{f_e}} - 1\right) \quad (16)$$

Prediction of change in $\mathbf{CL_{sec}}$ and $\mathbf{CL_{renal}}$ or $\mathbf{CL_{total}}$ of the training and verification drugs in pregnancy

The ratio of the predicted $\mathrm{CL}_{\mathrm{sec,B}}^{\mathrm{pregnant}}$, $\mathrm{CL}_{\mathrm{renal,B}}^{\mathrm{pregnant}}$ or $\mathrm{CL}_{\mathrm{total,B}}^{\mathrm{pregnant}}$ and the corresponding clearance in non-pregnant women (i.e. fold-change in different trimesters) was estimated for the verification and training drugs. The latter was estimated as indicated above for the verification drugs (Eq 11-16). That is, the small contribution of other OAT contributing to their secretion was taken into consideration.

Data analysis

To assess the accuracy of our predictions, the ratio of the predicted and the observed data, and absolute average fold error (AAFE) in the predictions of verification drugs were calculated as follows:

$$\mathrm{Ratio} = \frac{\mathrm{Predicted}}{\mathrm{Observed}}$$

$$\mathrm{AAFE} = 10^{\left|\frac{1}{\mathrm{N}}\sum \log\frac{\mathrm{predicted}}{\mathrm{observed}}\right|}$$

Our *a priori* acceptable range for the ratio was 0.8-1.25, *i.e.* the bioequivalence criteria.

Results

Pharmacokinetic data collection

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Where literature data were available, we estimated $\mathrm{CL}_{\mathrm{int,sec}}$ of tenofovir, acyclovir, oseltamivir carboxylate, amoxicillin, cefazolin, pravastatin and ketorolac, R- ketorolac, S- ketorolac in non-pregnant and pregnant women at various gestational ages (Table 1).

Fraction transported by $\mathrm{OAT_j}$ in the non-pregnant population

As expected, the training drugs, tenofovir, acyclovir and oseltamivir carboxylate were found to be selectively transported by OAT1, OAT2 and OAT3: $\mathrm{ft}_{\mathrm{OAT1}}$ of tenofovir, $\mathrm{ft}_{\mathrm{OAT2}}$ of acyclovir and $\mathrm{ft}_{\mathrm{OAT3}}$ of oseltamivir carboxylate were 94%, 99% and 100%, respectively (Table 2 and Supplementary Table 1). Regarding the verification dataset drugs, pravastatin was transported by OAT3, with a $\mathrm{ft}_{\mathrm{OAT3}}$ of 100%. Cefazolin was transported by OAT1 (3%) and OAT3 (97%), ketorolac and its isomers by OAT1 (72%) and OAT2 (29%), and amoxicillin by OAT1 (9%) and OAT3 (91%), respectively.

Fold-change in OAT activity during pregnancy

Compared to postpartum, OAT1 activity increased 2.9- and 1.0-fold in the 2nd and 3rd trimester, respectively, while OAT3 activity increased 2.2-, 1.7- and 1.3-fold in the 1st, 2nd and 3rd trimester, respectively. Compared to healthy individual, OAT2 activity increased 3.1 -fold in the 3rd trimester (Fig. 2).

Predicted and observed renal and secretory clearance values of the verification drugs

The predicted/observed ratio of $\mathrm{CL}_{\mathrm{sec,B}}^{\mathrm{pregnant}}$ of amoxicillin (2nd trimester), pravastatin and ketorolac, R-ketorolac, S-ketorolac fell within our *a priori* acceptance criteria (0.8-1.25 fold of the observed data) (Fig. 3, Table 3). The predicted/observed ratio of $\mathrm{CL}_{\mathrm{sec,B}}^{\mathrm{pregnant}}$ of amoxicillin in the 3rd

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trimester and cefazolin in the 2nd trimester did not. They were 0.76 and 1.27 (but within 1.5-fold error), respectively. $CL_{renal,B}^{pregnant}$ and $CL_{total,B}^{pregnant}$ of verification dataset were all well-predicted (within 0.80-1.25 fold, Fig. 3). The AAFE of $CL_{sec,B}^{pregnant}$, $CL_{renal,B}^{pregnant}$ and $CL_{total,B}^{pregnant}$ of the verification drugs for the 2nd trimester were 1.07, 1.02 and 1.04 respectively, and in the 3rd trimester were 1.05, 1.02 and 1.05, respectively.

Predicted change in CL_{sec} and CL_{renal} or CL_{total} of the training and verification drugs in pregnancy

As expected the total CL $\left(CL_{total,B}^{pregnant}\right)$ of an OAT-secreted drug is most affected by OAT induction in pregnancy when f_e of the drug is large, and when CL_{sec} is a large fraction of the total CL_{renal} of the drug (e.g. oseltamivir carboxylate, Fig.4, Supplementary Table 2).

Discussion

Here, we predicted the $CL_{sec,B}^{pregnant}$, $CL_{renal,B}^{pregnant}$ and $CL_{total,B}^{pregnant}$ of OAT drugs transported by single or multiple OATs. In doing so, we employed an experimental design not adopted by others when predicting renal CL of drugs (Coen Van Hasselt *et al.*, 2014; Wang *et al.*, 2019). First, this is the first time that probe drug data, in combination with the RAF approach, have been used to predict CL_{sec} and CL_{renal} of drugs during pregnancy. Others have reported using PBPK models to predict renal CL of OAT drugs during pregnancy. However, they have either ignored pregnancy-induced changed in renal secretion of these drugs or have concluded that such changes are absent (De Sousa Mendes *et al.*, 2016; Dallmann, Ince, Solodenko, *et al.*, 2017); second, we focused on predicting CL_{sec} rather that CL_{renal} or CL_{total} as the latter two can be predicted well even when CL_{sec} is poorly predicted such as for drugs where CL_{sec} is a small or a

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minor contributor to $\mathrm{CL_{renal}}$; third, most publications use creatinine renal CL as a measure of glomerular filtration CL (Banfi *et al.*, 2009; Garner *et al.*,2019; Wiles *et al.*, 2020). However, creatinine is also secreted via OCT2 and OAT2 (Gutiérrez *et al.*, 2014; Lepist *et al.*, 2014; Chu *et al.*, 2016) which could confound interpretation of changes in OAT/OCT activity during pregnancy. Therefore, we used inulin renal CL, a gold-standard measure of glomerular filtration CL, because it is not actively secreted (Koetje *et al.*, 2011; Shannon and Smith, 1953; Smith *et al.*, 2008).

As expected, the fraction of probe drugs transported by the OATs (training dataset) was more than 90% (Table 2). Further, there is no evidence of transporter-mediated reabsorption of tenofovir, acyclovir or oseltamivir carboxylate, and the $\mathrm{CL_{PD}}$ of these drugs is relatively small (Table 1). Therefore, tenofovir, acyclovir and oseltamivir carboxylate can be used with confidence as selective *in vivo* probes of pregnancy-induced changes in OAT1, OAT2 and OAT3 activity, respectively.

We found that each OAT activity was induced by pregnancy to a different extent, with maximum induction of about 3-fold for OAT1 and OAT2 and about 2-fold for OAT3 (Fig. 2). The time course of this induction also varied, with OAT3 activity peaking earlier (1st trimester) than OAT1 (2nd trimester) while OAT2 activity peaked in the 3rd trimester. However, additional data at various gestational ages are needed to confirm this conclusion. This pattern of change in OAT activity during pregnancy may be driven by the patten of changes in plasma concentration of pregnancy hormones as we have shown previously for induction of hepatic CYP3A4 (Zhang *et al.*, 2015). For example the plasma concentration of estradiol, progesterone, human chorionic gonadotropin (HCG) and relaxin peaks during 1st, 2nd and 3rd trimester of human pregnancy, respectively (Steroid Endocrinology of Pregnancy, 2009; Papageorgiou *et al.*, 2013; Cheung

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and Lafayette, 2013). Indeed, experiments with opossum kidney (OK) cells show that 17β-estradiol induces human renal OAT1 likely via the estrogen receptor α (ERα) (Euteneuer *et al.*, 2019). Expression of renal Oat2 mRNA level (slc22a7) increases 1.19 to 1.88- fold during pregnancy in mice (Aleksunes, 2013). And, mRNA expression of rat Oat2 is modestly upregulated by estradiol and progesterone (1.25 and 1.18-fold) but significantly down-regulated by testosterone (0.27-fold) (Ljubojević *et al.*, 2007). Last, the activity of OAT3 increased during pregnancy, and it was the highest in the 1st trimester. This trend was similar to the change in plasma concentration of HCG and relaxin (Cheung and Lafayette, 2013). These data of pregnancy-induced OAT induction are hypothesis-driving observations and should be followed up by experimental studies to determine the mechanism(s) of regulation of OAT transporters. An alternative explanation is that this OAT induction is a non-specific chage in the size and volume of kidneys and/or the length of proximal tubule during pregnancy (Cheung and Lafayette, 2013). However, such a change would likely result in the same extent and time-frame of induction of the OATs. Our data suggest that this is not the case (Fig. 2).

Based on the changes in OAT activity during pregnancy, we successfully predicted the pregnancy-induced changes in $\mathrm{CL}_{\mathrm{sec,B}}^{\mathrm{pregnant}}$, $\mathrm{CL}_{\mathrm{renal,B}}^{\mathrm{pregnant}}$ and $\mathrm{CL}_{\mathrm{total,B}}^{\mathrm{pregnant}}$ of the verification drugs (amoxicillin, cefazolin, pravastatin and ketorolac (R- ketorolac, S- ketorolac and the racemic mixture)) (Table 3, Fig. 3). These predictions were within our *a priori* acceptance criteria (i.e. 0.80-1.25 fold of the observed value), except for $\mathrm{CL}_{\mathrm{sec,B}}^{\mathrm{pregnant}}$ of amoxicillin in the 3rd trimester and cefazolin in the 2nd trimester, which were marginally outside (0.76 and 1.27, respectively) our acceptance criteria (Fig. 3). This highlights the importance of predicting the $\mathrm{CL}_{\mathrm{sec,B}}^{\mathrm{pregnant}}$ rather than $\mathrm{CL}_{\mathrm{renal,B}}^{\mathrm{pregnant}}$ or $\mathrm{CL}_{\mathrm{total,B}}^{\mathrm{pregnant}}$ of drugs.

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There are a few limiations to our study. First, we assumed that the rate-determining step in renal secretory CL of the drugs was their OAT-mediated transport. That is, even though some of the probe and verification drugs are substrates of other transporters (basal and apical; see Supplementary Table 1), we assumed that their secretory CL was determined only by OATs. Given the low CL_{PD} of the drugs, and assuming this value applies to the basal efflux CL of the drugs, based on the extended CL model, this is a resonable assumption (Patilea-Vrana and Unadkat, 2016). If this assumption is incorrect, then the proposed approach will work only if probe drugs are available that selectively report the activity of each of these alternative transporters. Second, we assumed that $\mathrm{CL}_{\mathrm{non-renal}}$ of tenofovir, acyclovir, oseltamivir carboxylate, cefazolin and ketorolac are not affected by pregnancy. About 20% of ketorolac is metabolized by UGT enzyme and 9-14% of acyclovir is metabolized by alcohol and aldehyde dehydrogenases. But, these enzymes may not be affected by human pregnancy (Mroszczak *et al.*, 1987; De Miranda and Good, 1992; Anderson, 2005; Jelski *et al.*, 2020). Third, the pharmacokinetics of the drugs in postpartum women were assumed to have returned to those in non-pregnant women prior to pregnancy. Indeed, comparison of their postpartum pharmacokinetics with those in non-pregnant individuals, supported this conclusion (data not shown). Fourth, $\mathrm{F}_{\mathrm{reabs}}$ was assumed to be negligible as previously justified (Mathialagan *et al.*, 2017). If this assumption is substantially incorrect, our predictions for the verification drugs would not have met our acceptance criterion. Last, but not least, *in vivo* data for OAT1 in the 1st trimester or for OAT2 in the 1st and 2nd trimester are, as yet, not available, limiting the application of our approach to OAT1 and OAT2 substrates in these trimesters.

Based on the above observations, an important question that arises is: under what circumstances will pregnancy-induced changes in OAT-mediated drug CL result in recommendation of change in dosing regimen of a drug administered to pregnant women? Of

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course, the drug would have to be predominately renall cleared from the body and CL_{sec} would need to constitute a large fraction of CL_{renal} of the drug (high CL_{sec} /CL_{renal} and therefore low $fu_B * GFR_{inulin}/CL_{renal,B}$). A good example of these guidelines is acyclovir (Fig. 4 and Supplementary Table 2). Acyclovir's f_e is 0.76 and CL_{sec} /CL_{renal} is 0.57 and it is secreted predomnately by OAT2 (97%) which is induced by 3.1-fold during the 3rd trimester of pregnancy. Based on these data, we predict that its CL_{total} will increase (compared with non-pregnant population) by 1.69-fold during the 3rd trimester. However, if acyclovir's f_e and CL_{sec} /CL_{renal} were much greater (e.g. 0.9), the change in the CL_{total} of this hypothetical drug would be 2.4-fold. Such a change would warrant dosing regimen change for **only** those drugs that have a narrow therapeutic window. In contrast, a small CL_{sec} /CL_{renal} or f_e will reduce the impact of OAT induction on CL_{total} of the drug in pregnant women (Fig. 4). For example, the predicted CL_{total} of tenofovir in the 2nd trimester was less affected by induction of OAT1 activity (2.9-fold), even though its f_e is 0.81, because its CL_{sec} /CL_{renal} is small (0.23). This is not surprising since contribution by filtration clearance modulates the change in CL_{renal} produced by large and significant changes in CL_{sec} of the drug. For example, the maximum reduction in *in vivo* CL_{total} of an OAT substrate (bumetanide) in the presence of probenecid, a potent OAT inhibitor, is ~85% (Mathialagan *et al.*, 2017).

In summary, we showed for the first time that the changes in $CL_{sec,B}^{pregnant}$ and $CL_{renal,B}^{pregnant}$ of OAT-transported drugs can be predicted during pregnancy using probe drugs and the RAF approach. This approach could be used in the future to prospectively adjust dosing regimens of renally secreted OAT drugs (likely narrow therapeutic window drugs) administered to pregnant women without a need to conduct pharmacokinetic studies in this difficult to study and understudied population.

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Authorship Contributions

Participated in research design: Peng, Ladumor and Unadkat.

Conducted experiments: Peng and Ladumor.

Performed data analysis: Peng, Ladumor and Unadkat.

Wrote or contributed to the writing of the manuscript: Peng, Ladumor and Unadkat.

Reference:

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Footnotes

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Figure Legends

Fig. 1. Workflow for predicting $\mathbf{CL_{sec,B}^{pregnant}}$ and $\mathbf{CL_{renal,B}^{pregnant}}$ of the verification drugs based on *in vivo* renal CL of the probe drugs during pregnancy. # **-** *In vivo* $\mathrm{CL_{int,PD}}$ of the drugs was estimated from previously reported *in vitro* $\mathrm{CL_{int,PD}}$, MPPGK and kidney weight (Eq. 7), MPPGK (i.e. milligram of total proteins per gram of kidney) was 15 mg/g and kidney weight was 4.3 g/kg body weight (Mathialagan *et al.*, 2017). $ - fold-change in OAT activity during pregnancy was calculated using Eq. 8. $\mathrm{ft_{OAT\,j}}$ is the fractional contribution of each OAT of the drug in non-pregnant women where $\mathrm{OAT_j}$ indicates OAT1, OAT2 or OAT3. RAF for OAT1, OAT2, and OAT3 was 0.64, 7.32 and 4.09, respectively (Mathialagan *et al.,* 2017).

Fig. 2. Fold-change in *in vivo* $\mathbf{OAT_j}$ activity at various stages of pregnancy. Fold-change in *in vivo* OAT activity due to pregnancy (pregnant/postpartum or pregnant/non-pregnant population) as measured by the change *in vivo* $\mathrm{CL_{int,\,OATj}}$ of tenofovir (OAT1), acyclovir (OAT2), and oseltamivir carboxylate (OAT3), respectively.

Fig. 3. Predicted *in vivo* (A) $\mathbf{CL_{sec,B}^{pregnant}}$, (B) $\mathbf{CL_{renal,B}^{pregnant}}$ or (C) $\mathbf{CL_{total,B}^{pregnant}}$ of the verification drugs show good to excellent agreement with the observed data. The predicted $\mathrm{CL_{sec,B}^{pregnant}}$ of amoxicillin (2nd trimester), pravastatin and ketorolac, R-ketorolac, S-ketorolac fell within our *a priori* acceptance criteria (0.8-1.25 fold of observed values; dotted blue lines). However, the predicted/observed ratio of $\mathrm{CL_{sec,B}^{pregnant}}$ of amoxicillin (3rd trimester) and cefazolin (2nd trimester) did not. They were 0.76 and 1.27 of the observed values, respectively. In contrast, the predicted $\mathrm{CL_{renal,B}^{pregnant}}$ and $\mathrm{CL_{total,B}^{pregnant}}$ of the verification drug all fell within our *a priori* acceptance criteria (within 0.8-1.25 fold of observed values).

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Fig. 4. Predicted fold-change in $\mathbf{CL_{sec,B}^{pregnant}}$, $\mathbf{CL_{renal,B}^{pregnant}}$ and $\mathbf{CL_{total,B}^{pregnant}}$ of the training and verification drugs when compared with that in the non-pregnant population. The CL_{total} of a drug was most affected by induction of OAT when f_e and CL_{sec}/CL_{renal} of the drug are large (e.g. oseltamivir carboxylate).

Table 1 Summary of PK data of training and verification drugs

	f_e [a]	F[a]	fu[a]	BP[a]	$CL_{int,sec}$ (mL/min/kg)				$CL_{int,PD}$		reference
					non pregnant	1st trimester [b]	2nd trimester	3rd trimester	*in vitro* (uL/min/mg protein)	*in vivo* (mL/min/kg) [c]	
Tenofovir	0.81	0.25	0.99	0.58	0.49,0.57	NA	1.39	0.43,0.65	0.10	0.01	(Best et al., 2015) (Colbers et al., 2013)
Acyclovir	0.76	0.22	0.85	1.00	2.80	NA	NA	8.66	0.27	0.02	(De Miranda and Blum, 1983) (Kimberlin et al., 1998)
Oseltamivir carboxylate	0.93	0.79	0.97	0.67	3.43	7.45	5.70	4.39	0.10	0.01	(Pillai et al., 2015)
Amoxicillin	0.58	0.93	0.85	0.67	3.68	NA	7.29	6.85	4.60	0.36	(Andrew et al., 2007)
Cefazolin	0.80	1.00	0.18	0.55	4.40	NA	5.63	NA	1.70	0.11	(Philipson et al., 1987)
Pravastatin	0.45	0.18	0.47	0.56	13.51	NA	19.82	22.94	0.16	0.01	(Costantine et al., 2016)
Ketorolac	0.60	1.00	0.01	1.00	46.94	NA	NA	64.30	15.80	1.02	(Kulo et al., 2012)
S-ketorolac	0.60	1.00	0.01	1.00	62.51	NA	NA	103.68	15.80	1.02	(Kulo et al., 2017)
R-ketorolac	0.60	1.00	0.01	1.00	38.30	NA	NA	49.63	15.80	1.02	(Kulo et al., 2017)

a F, f_e, fu and BP of the drugs are from nonpregnant women, except acyclovir where the data are from healthy individuals (men and women).

b 1st trimester: gestational age 1-12 weeks, 2nd trimester: gestational age 13-28 weeks, 3rd trimester: gestational age 29-40 weeks

c *In vivo* $CL_{int,PD}$ were estimated from *in vitro* $CL_{int,PD}$ (Mathialagan *et al.*, 2017).

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Table 2. Estimates of the fraction of drug transported by each OAT, *in vivo*, in the non-pregnant population

Dataset	Drugs	$ft_{OAT\,1}$(%)	$ft_{OAT\,2}$(%)	$ft_{OAT\,3}$(%)
Training dataset	Tenofovir	94.00	0.00	6.00
	Acyclovir	0.00	99.00	1.00
	Oseltamivir carboxylate	0.00	0.00	100
Verification dataset	Pravastatin	0.00	0.00	100
	Cefazolin	3.00	0.00	97.00
	Amoxicillin	9.00	0.00	91.00
	Ketorolac (R-, S- and racemic mixture)	71.00	29.00	0.00

Fraction of drug transported *in vivo* by OATj ($ft_{OAT\,j}$) $= \frac{RAF_{OATx} * CL_{int,\,OATj}}{\sum_{3}^{1} RAF_{OATx} * CL_{int,OATj}}$, where RAF is 0.64, 7.32 and 4.09 for OAT1, OAT2, and OAT3, respectively (Mathialagan *et al.*, 2017). The *in vitro* clearances ($CL_{int,OATj}$) were obtained from a previous study (Mathialagan *et al.*, 2017). $ft_{OAT\,j}$ of R- ketorolac and S- ketorolac are assumed to be the same as the racemic mixture of ketorolac

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Table 3. Predicted and observed values of $CL_{sec,B}^{pregnant}$, $CL_{renal,B}^{pregnant}$ and their corresponding ratios for the verification drugs

Drug (transporters)	Parameters	$CL_{sec,B}^{pregnant}$ (mL/min/kg)		$CL_{renal,B}^{pregnant}$ (mL/min/kg)		Reference
		2nd trimester [c]	3rd trimester [c]	2nd trimester	3rd trimester	
Ketorolac (OAT1,2)	Observed	NA	0.59	NA	0.61	(Kulo *et al.*, 2012)
	predicted	NA	0.69	NA	0.71	
	ratio [a]	NA	1.17	NA	1.16	
S-ketorolac (OAT1,2)	Observed	NA	1.14	NA	1.17	(Kulo *et al.*, 2017)
	predicted	NA	1.11	NA	1.14	
	ratio	NA	0.97	NA	0.97	
R-ketorolac (OAT1,2)	Observed	NA	0.35	NA	0.36	(Kulo *et al.*, 2017)
	predicted	NA	0.43	NA	0.44	
	ratio	NA	1.23	NA	1.22	
Pravastatin (OAT3)	Observed	8.65	8.49	10.54	10.42	(Costantine *et al.*, 2016)
	predicted	9.19	7.48	11.08	9.40	
	ratio	1.06	0.88	1.05	0.90	
Amoxicillin (OAT1,3)	Observed	5.96	5.37	8.63	8.05	(Andrew *et al.*, 2007)
	predicted	5.41	4.07	8.09	6.75	
	ratio	0.91	0.76	0.94	0.84	
Cefazolin (OAT1,3)	Observed	1.85	NA	2.64	NA	(Philipson *et al.*, 1987)
	predicted	2.35	NA	3.14	NA	
	ratio	1.27	NA	1.19	NA	
	AAFE [b]	1.07	1.02	1.05	1.02	

a - $\text{ratio} = \frac{\text{Predicted}}{\text{Observed}}$

b - absolute average fold error $(\text{AAFE}) = 10^{|\frac{1}{N}\sum \log\frac{\text{predicted}}{\text{observed}}|}$

c - 2nd trimester: gestational age 13-28 weeks, 3rd trimester: gestational age 29-40 weeks

NA- not available

Figure 1

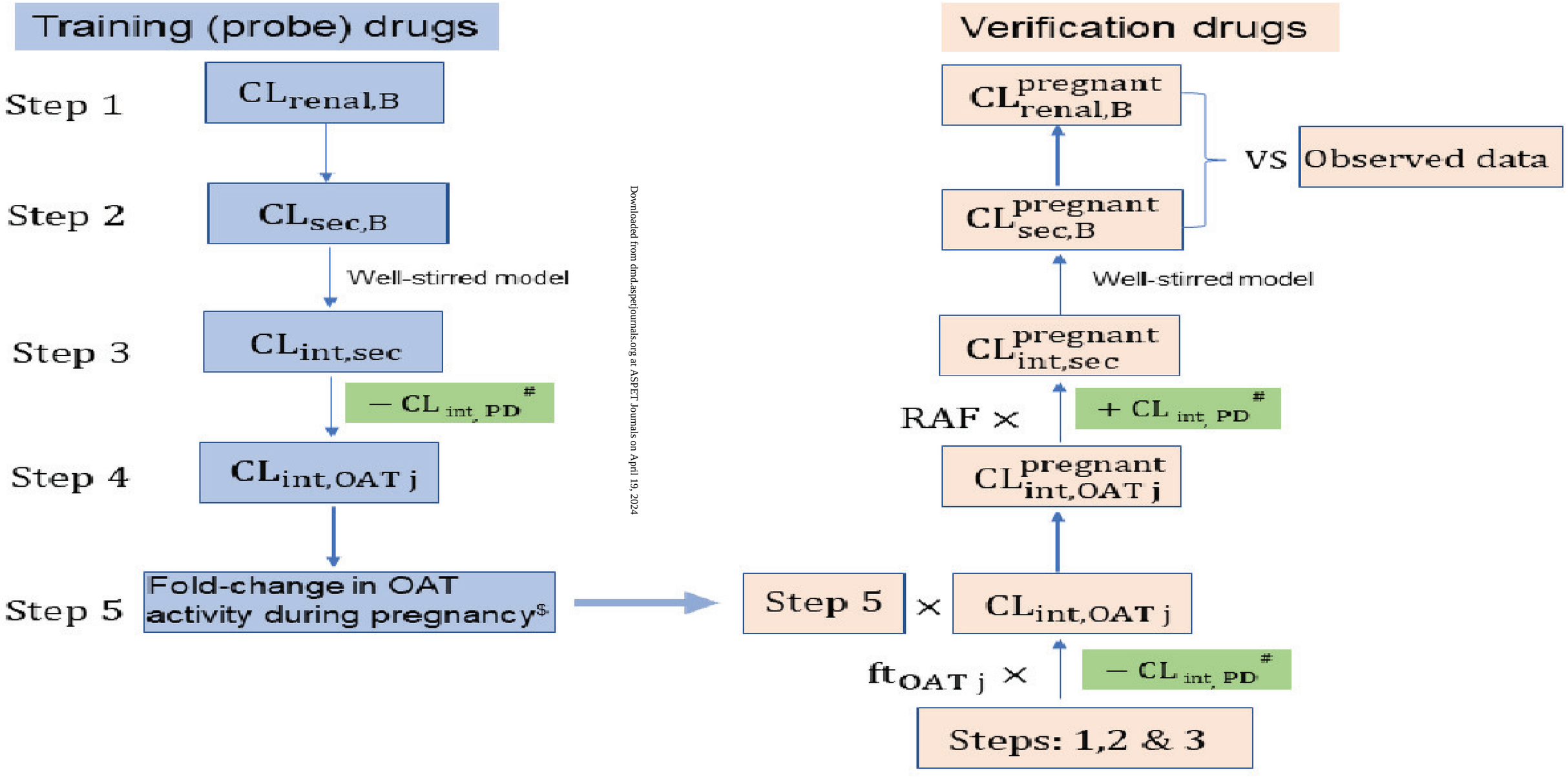

Figure 2

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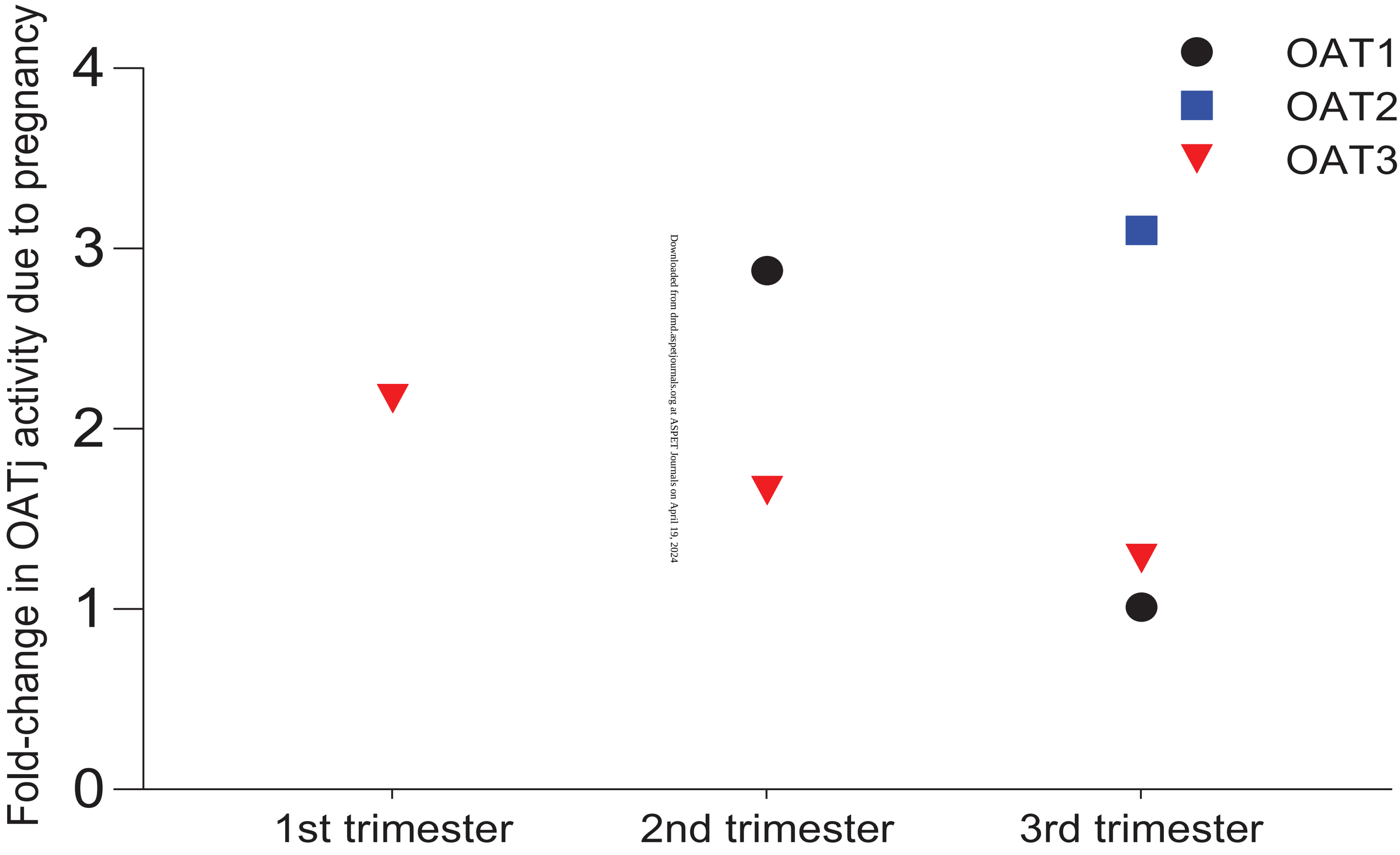


Figure 3

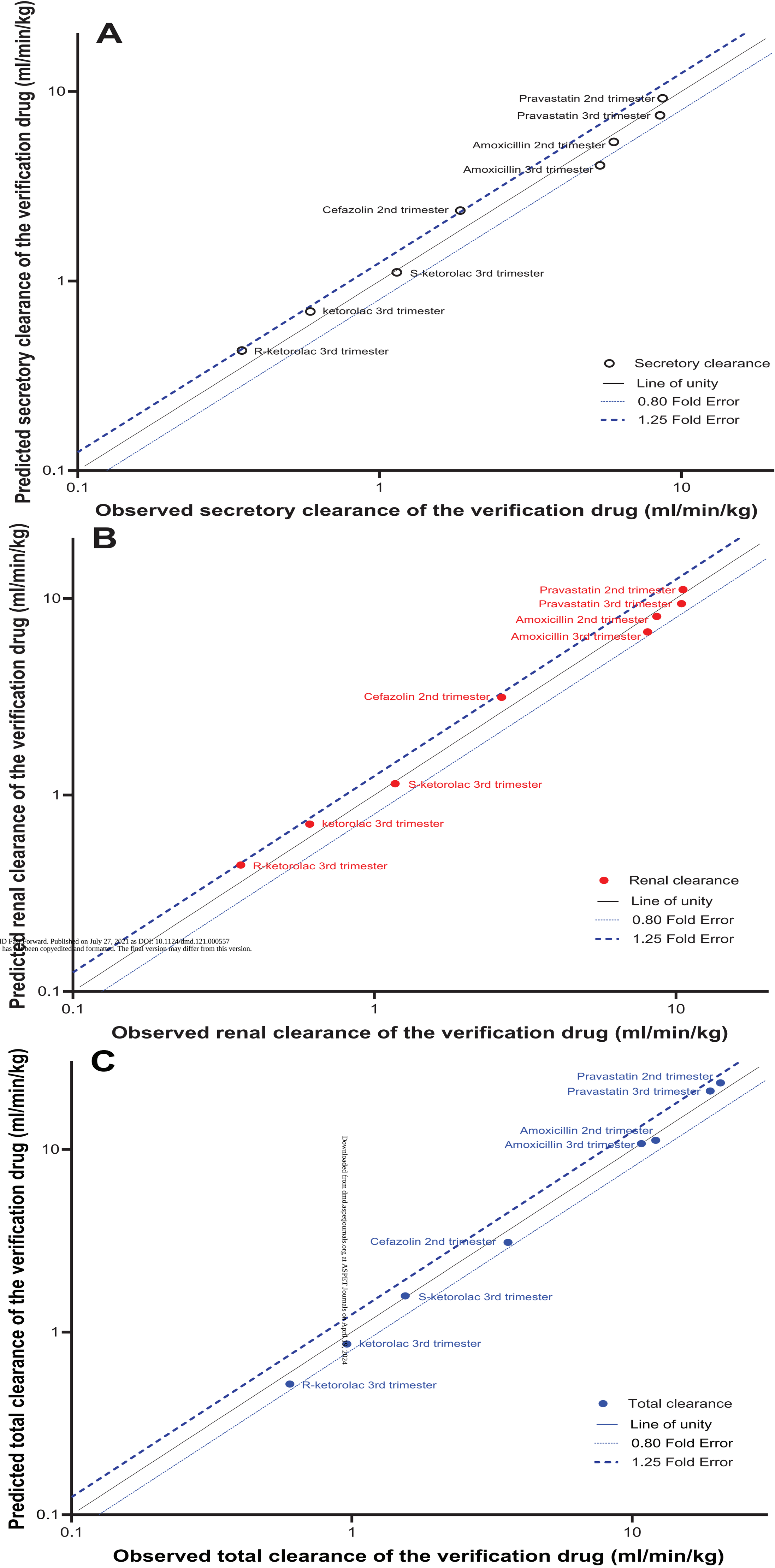
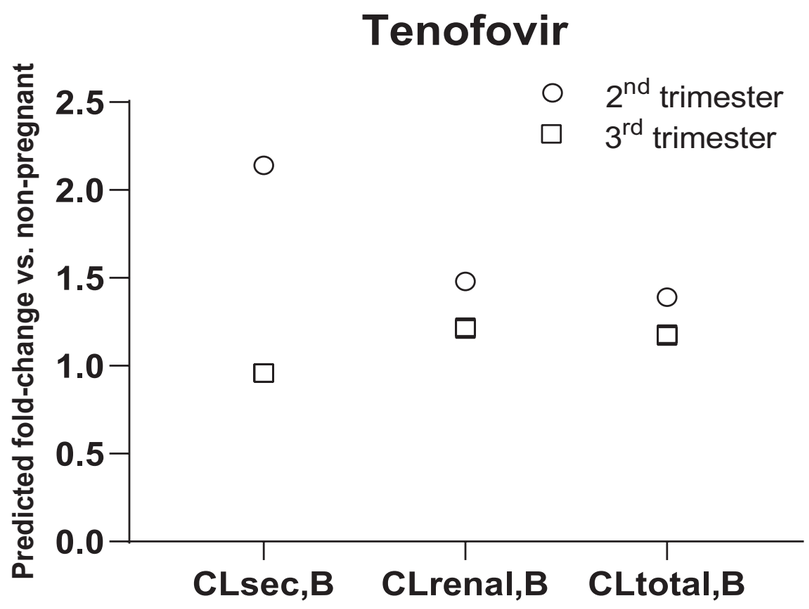
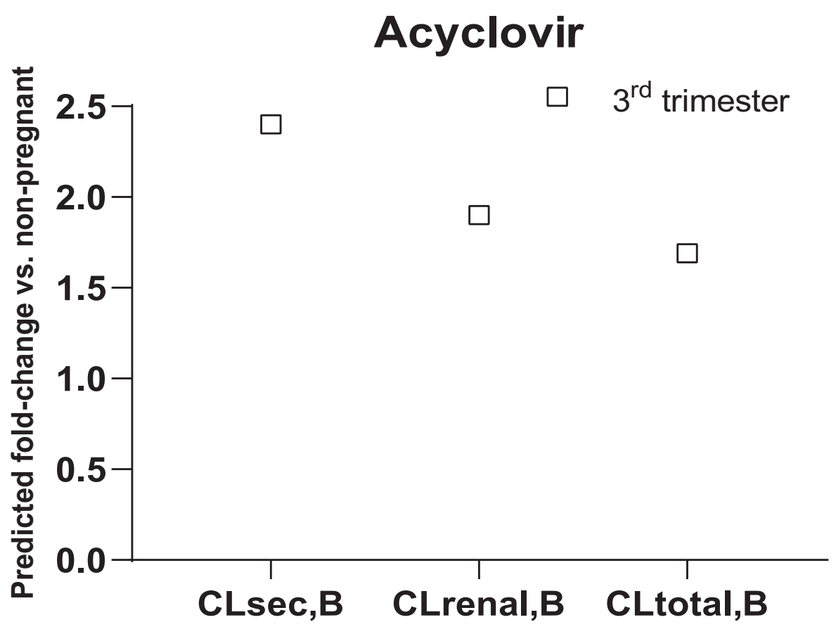
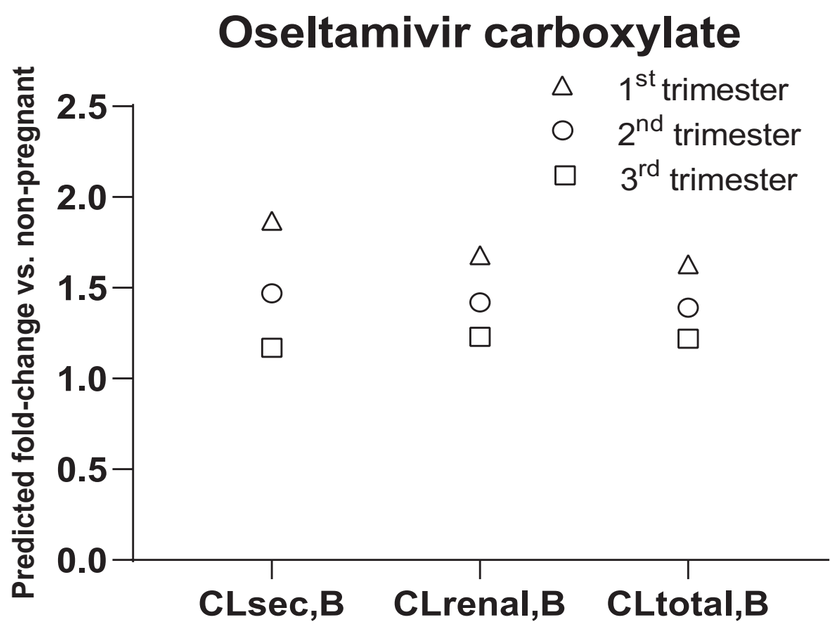


Figure 4







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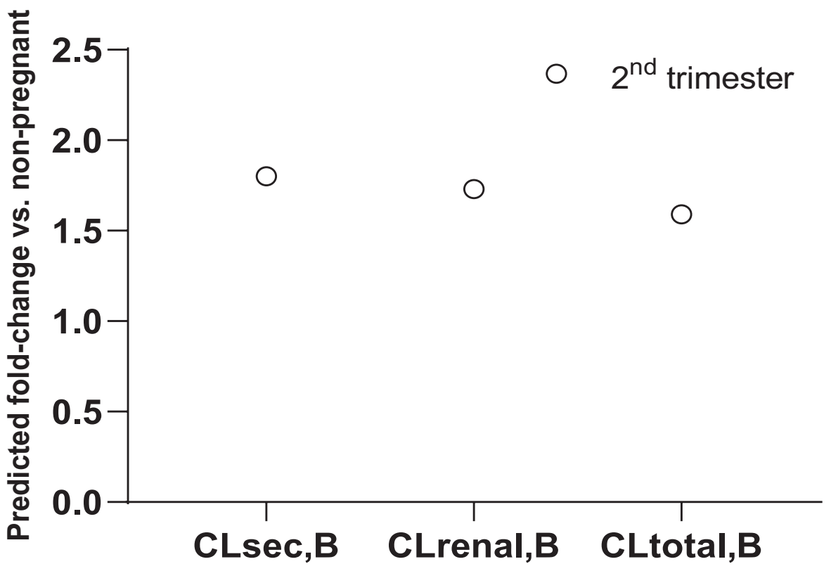

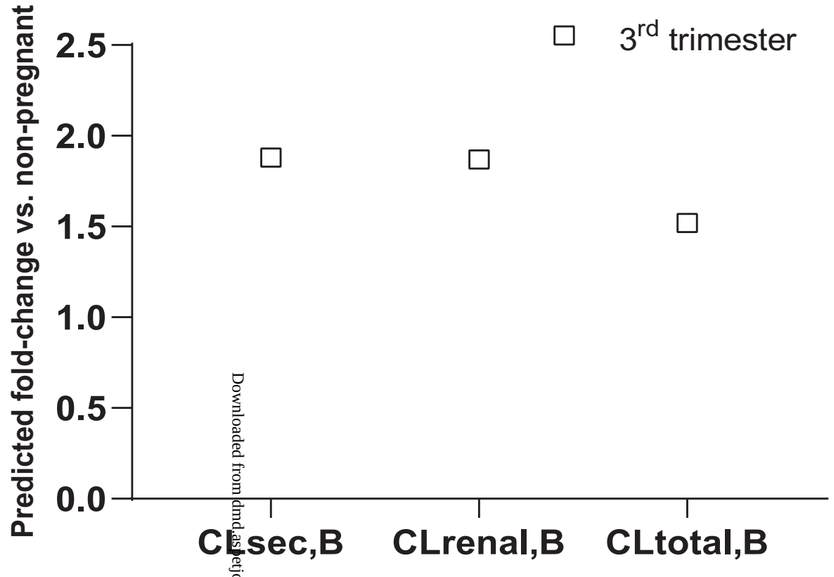

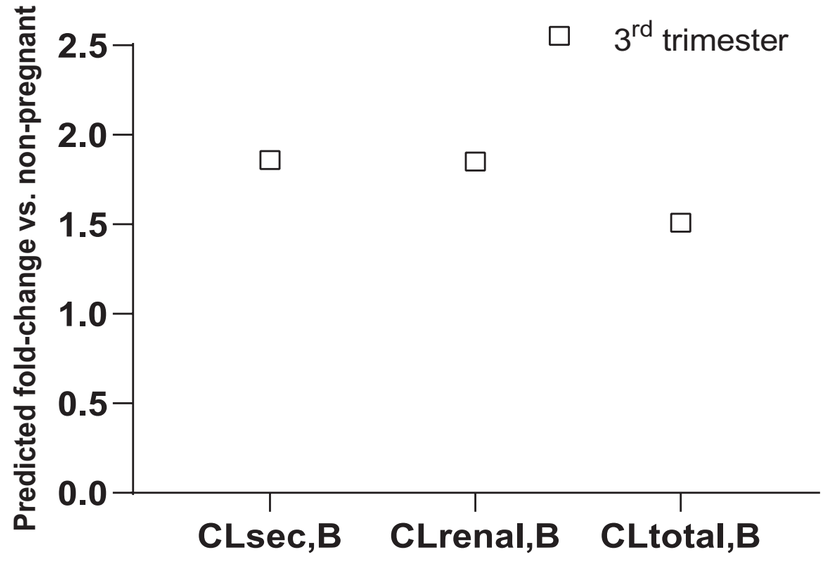

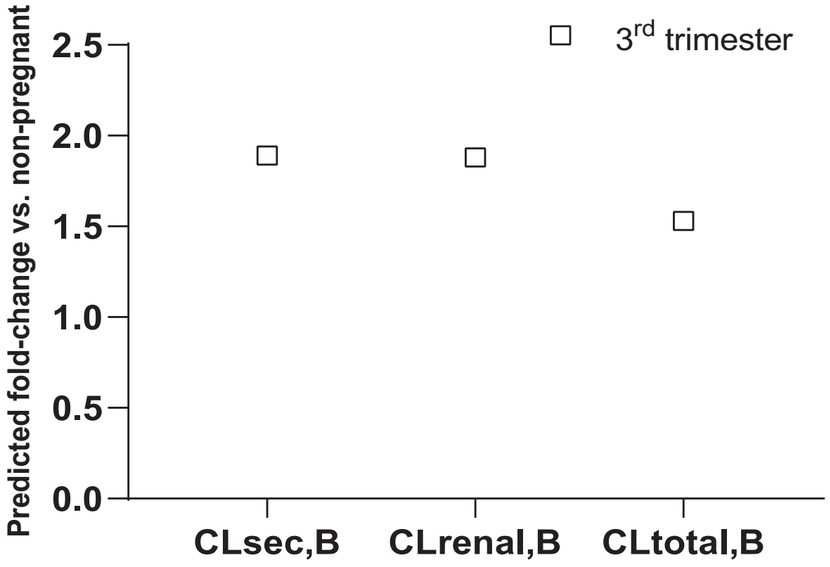

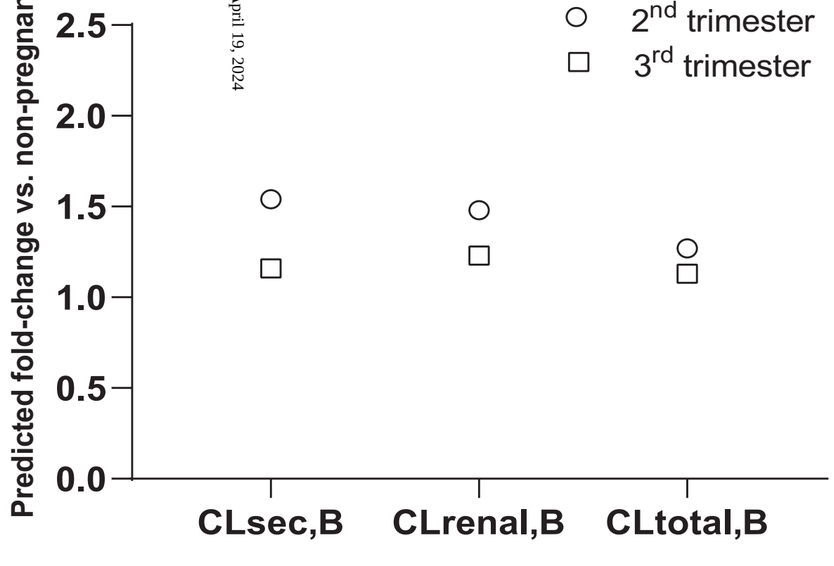

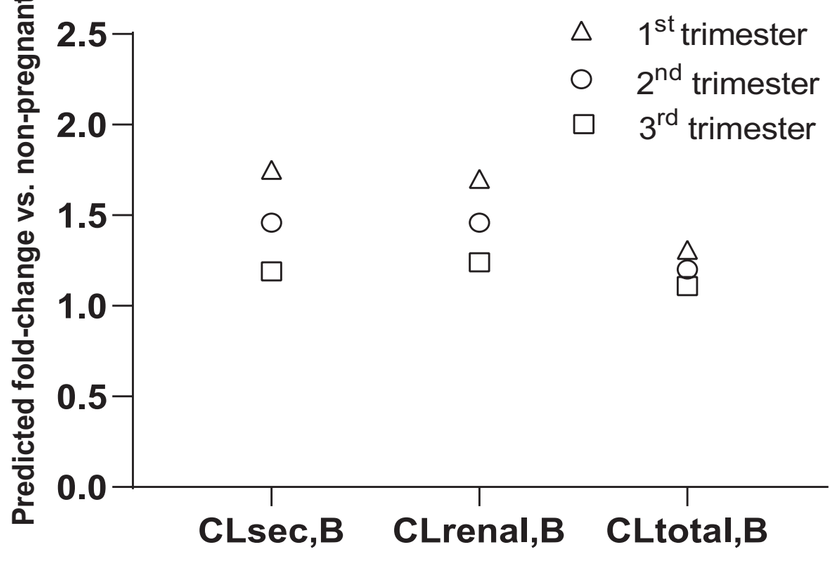

Supplementary Information (DMD-AR-2021-000557R1)

Prediction of pregnancy-related changes in secretory and total renal clearance of drugs transported by organic anion transporters

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Supplementary Table 1. Summary of renal transporters known to transport the probe and verification drugs*

Drugs	Basal secretion			Apical secretion					Apical reabsorption		Reference
	OAT1	OAT2	OAT3	MATE1/2k	P-gp	MRP2	MRP4	BCRP	OAT4	PEPT1/2	
Tenofovir	Yes	No			No	No	Yes				(Ray *et al.*, 2006; Imaoka *et al.*, 2007; Uwai *et al.*, 2007; Kohler *et al.*, 2011; Furihata *et al.*, 2017; Mathialagan *et al.*, 2017)
Acyclovir	Yes	Yes (primary)	Yes	Yes				Yes			(Takeda *et al.*, 2002; Tanihara *et al.*, 2007; Gunness *et al.*, 2011; Cheng *et al.*, 2012; Nies *et al.*, 2012; Ye *et al.*, 2012, 2013; Mathialagan *et al.*, 2017)
Oseltamivir carboxylate			Yes		No		Yes			No	(Morimoto *et al.*, 2008; Ose *et al.*, 2009; Poirier *et al.*, 2012; Mathialagan *et al.*, 2017)
pravastatin			Yes		No	Yes			Yes		(Maki *et al.*, 2002; Sakaeda *et al.*, 2002; Khamdang *et al.*, 2004; Matsushima *et al.*, 2005; Niemi *et al.*, 2006; Watanabe *et al.*, 2011; Ellis *et al.*, 2013; Mathialagan *et al.*, 2017; Huang *et al.*, 2018)
Cefazolin	Yes		Yes				Yes	Yes			(Uwai *et al.*, 2002; Sakurai *et al.*, 2004; Ueo *et al.*, 2005; Ci *et al.*, 2007; Kato *et al.*, 2008; Mathialagan *et al.*, 2017)
Amoxicillin	Yes		Yes							Yes	(Li *et al.*, 2006; Mathialagan *et al.*, 2017; Parvez *et al.*, 2018)
ketorolac	Yes	Yes									(Mathialagan *et al.*, 2017)

* - while these *in vitro* studies identified the transporters involved, their contribution *in vivo* may not necessarily be significant. This can only be assessed through studies presented here or other *in vitro* to *in vitro* scaling approaches.

Supplementary Table 2. Predicted fold-change in $CL_{sec,B}^{pregnant}$, $CL_{renal,B}^{pregnant}$ or $CL_{total,B}^{pregnant}$ of the training and verification drugs in pregnancy

Drug (transporters)	f_e[a]	$fu_B * GFR_{inuli}/CL_{renal.B}$[a]	CL_{sec}/CL_{renal}[a]	Fold-change in $CL_{sec,B}^{pregnant}$			Fold-change in $CL_{renal,B}^{pregnant}$			Fold-change in $CL_{total,B}^{pregnant}$		
				1st trimester	2nd trimester	3rd trimester	1st trimester	2nd trimester	3rd trimester	1st trimester	2nd trimester	3rd trimester
Tenofovir (OAT1)	0.81	0.77	0.23		2.14	0.96		1.48	1.22		1.39	1.18
Acyclovir (OAT2)	0.76	0.43	0.57			2.40			1.90			1.69

relative to that in non-pregnant individuals

Oseltamivir carboxylate (OAT3)	0.93	0.38	0.62	1.87	1.47	1.17	1.68	1.42	1.23	1.63	1.39	1.22
Cefazolin (OAT1,3)	0.80	0.28	0.72		1.80			1.73			1.59	
Ketorolac (OAT1,2)	0.60	0.03	0.97			1.88			1.87			1.52
S-ketorolac (OAT1,2)	0.60	0.03	0.97			1.86			1.85			1.51
R-ketorolac (OAT1,2)	0.60	0.04	0.96			1.89			1.88			1.53
Amoxicillin (OAT1,3)	0.58	0.36	0.64		1.54	1.16		1.48	1.23		1.27	1.13
Pravastatin (OAT3)	0.45	0.17	0.83	1.77	1.46	1.19	1.71	1.46	1.24	1.32	1.20	1.11

a f_e, fu_B and GFR_{inuli} are for nonpregnant women.

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