

Objectives

- Characterize the PK of ursodiol in newborn.
- Demonstrate the usefulness of AMS as a tool for studying PK in neonates
- Create a PhysioPD model of bile acid trafficking in infants.
- Compare the results from the PK/PD and PhysioPD models

Background

Pediatric Drug Development

Children are physiologically different from adults; therefore, drug studies conducted in adult populations cannot be simply extrapolated to pediatric populations. Many new drug applications still do not adequately address pediatric assessment, and little or no pediatric data exists for drugs approved before the Pediatric Research Equity Act (PREA). As a result, most medicines are prescribed to children in an off-label manner, with dosages extrapolated from adult data through body weight and surface-area calculations. This lack of pharmacokinetic information can result in adverse effects due to high doses, or suboptimal benefit due to inadequate doses.

Cholestasis

Cholestasis (reduction of the normal flow of bile from the liver to the small intestine) is a common affliction of premature neonates admitted to the neonatal intensive care unit. Ursodiol (Actigal®) is an endogenously produced bile acid approved to treat cholestasis in adults is frequently used off-label to treat neonatal cholestasis.

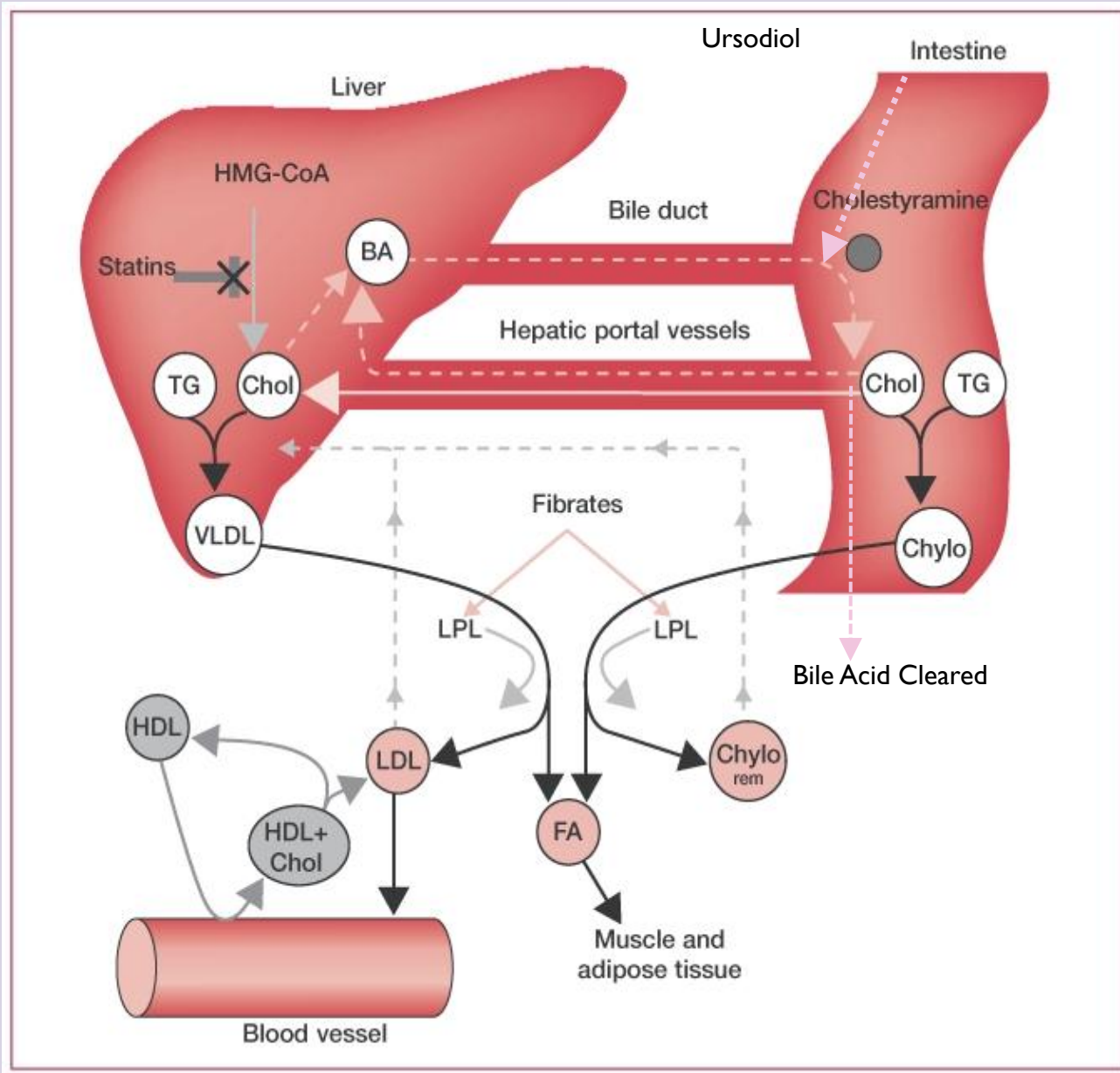


Figure 1. Graphical representation of bile trafficking. Ashley and Niebauer (2004) 5. Coronary artery disease. Cardiology Explained. London, Remedica. [cited 8/4/2010].

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The mechanism of action of ursodiol for treatment of cholestasis in neonates is unknown. Its efficacy varies widely from patient to patient. The PK of UDCA in neonates, which may explain its wide variation in efficacy, have never been characterized because measurement requires differentiating between endogenous and exogenous compound by using a labeled tracer. This is the first clinical study of UDCA PK in neonates.

Ursodiol (ursodeoxycholic acid) is a normal part of human bile

Ursodiol is used to treat

- Gallstones
- Primary biliary cirrhosis
- Biliary atresia
- Parenteral nutrition-associated cholestasis

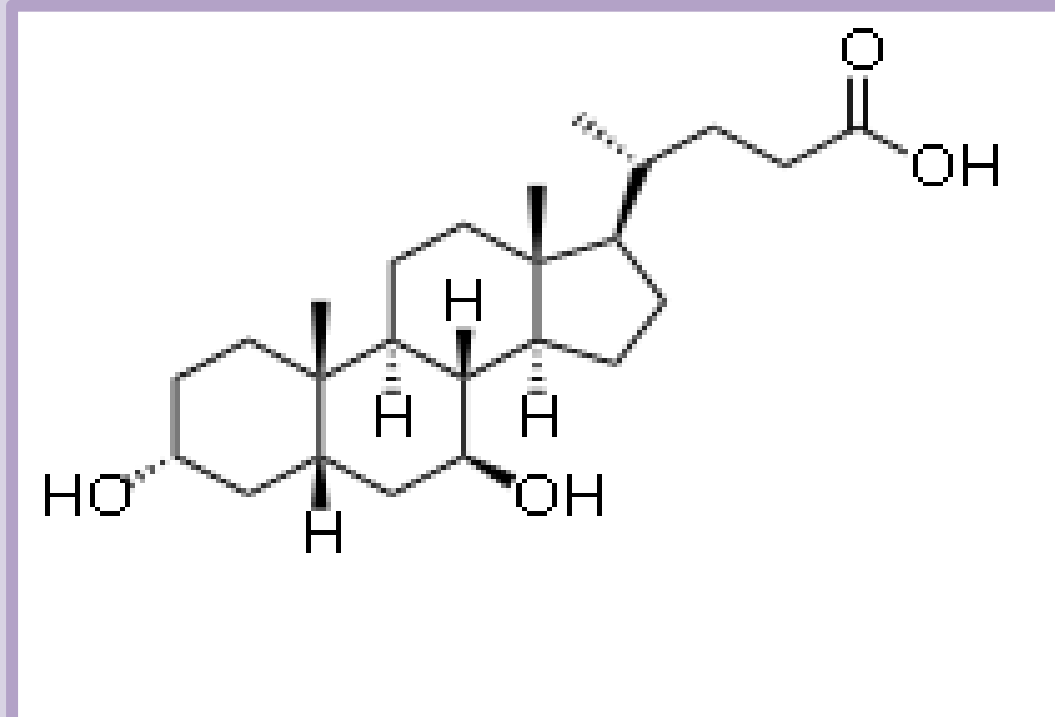


Figure 2. Structure of Ursodiol

In adults, Ursodiol functions by

- Inhibiting cholesterol absorption
- Suppressing cholesterol synthesis, secretion
- Solubilizing cholesterol
- Reducing bile viscosity
- Increasing bile flow

Clinical Trial

This Study was approved by the Loma Linda University Institutional Review Board and FDA-registered Radioactive Drug Research Committee.

The study drug was synthesized in a radiochemistry laboratory (Moravek Biochemicals).

Eligibility Criteria:

- Neonates admitted to the LLUCH Neonatal Intensive Care Unit
- No cholestasis
- Weight \geq 1,900 g
- Vascular indwelling catheter for multiple blood draws
- Nasogastric tube in place
- Hemoglobin $>$ 11 g/dL

Table 1. Subject demographics

Patient Demographics	(n=5)
Gestational Age (weeks)	36 (35-40)
Weight at Study Entry (grams)	2,755 (1,910-3,180)
Height (cm)	45 (42-47)
Body Surface Area (m ²) Haycock et al.	0.189 (0.151-0.208)
Gender (n - M/F)	5 (3/2)
Age at Study Entry (days)	2 (1-6)

Exclusion Criteria:

- Major gastrointestinal congenital anomalies
- Neonatal hepatitis
- Anatomic evidence of bile duct obstruction (biliary atresia)
- Cholelithiasis
- Symptoms suggestive of cystic fibrosis

Study Protocol:

- ¹⁴C-ursodiol was administered to neonates via NG tube in three different doses (1, 3.3, or 10 nanoCuries of radioactivity; 8, 26, or 80 nanograms of ursodiol) separated by intervals of 48 hrs.
- Blood samples (0.25 ml each) were drawn \leq 0.5 hrs before each dose administration, then again at 0.5, 1.5, 3, 6, 12, and 24 hrs after each dose
- Blood samples were centrifuged to separate serum from RBCs and then stored separately at -80° C until AMS analysis.
- Patient 4 was withdrawn from the study after the 2nd dose due to discharge, and Patient 5 was withdrawn after the 2nd dose due to withdrawn parental consent.

Pharmacokinetic Analysis

- Non-compartmental analysis was conducted in PK solver.
- Compartmental analysis was conducted in Nonmem.
- PhysioPD models were built using JDesigner in the Systems Biology Workbench. Data for the model were compiled from literature¹⁻³.

Results

Non-compartmental Analysis

Non-compartmental analysis (NCA) was conducted on the drug concentration data for the 8 ng dose. Each subject was evaluated separately and the results for all five subjects included in the analysis. A large variability was observed in the estimated parameters.

Table 2. NCA results

Parameter	Unit	Median	Mean	SD
t _{1/2}	h	42.95	44.13	14.15
t _{max}	h	3.00	2.70	2.17
C _{max}	ng/L	6.08	7.37	6.21
t _{lag}	h	0	0	0
AUC 0-inf	pg/ml·h	226.45	417.72	452.79
V _{Z/F}	L	1.96	2.05	1.19
CL/F	L/hr	0.035	0.038	0.026

Data Analysis

Initial evaluation of the data showed that while the doses administered were extremely small, the lowest measured drug concentration was significantly higher than the lower limits of quantification (LLOQ). This indicates that the total amount of labeled drug administered in future studies can be reduced, thus lowering the label exposure in newborns. Furthermore, smaller sample volumes may be needed in future clinical studies.

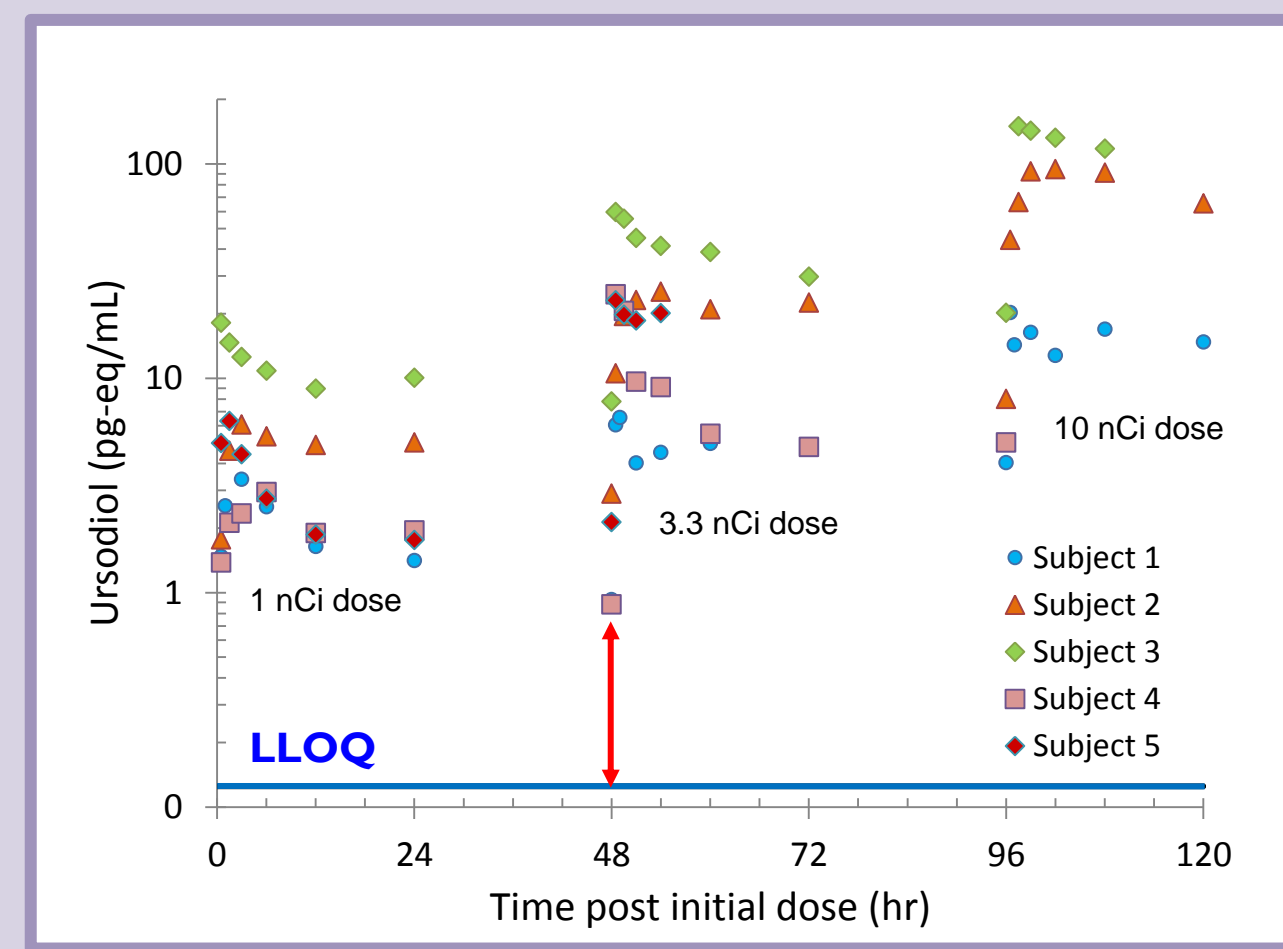


Figure 4. Ursodiol concentration compared to lower limit of quantification. Difference is shown as a red arrow.

Compartmental Analysis

Table 3. Population PK parameters

Parameter	Estimate (%RSE)	IIV (%RSE)
ka (h ⁻¹)	1.65 (0.76)	-
CL (L/hr)	0.022 (0.39)	0.76 (0.68)
V _p (L)	0.935 (0.94)	1.2 (0.9)
Q (L/h)	1.24 (0.76)	-
V ₂ (L)	1.21 (0.19)	0.86 (0.5)

Mixed effect modeling identified a 2-compartment model as the best fit of the data, as can be seen in various diagnostic graphs below. Furthermore, there was a generally good agreement with the NCA results of apparent CL and V values. Similar to the NCA results, large interindividual variability in the PK parameters was identified.

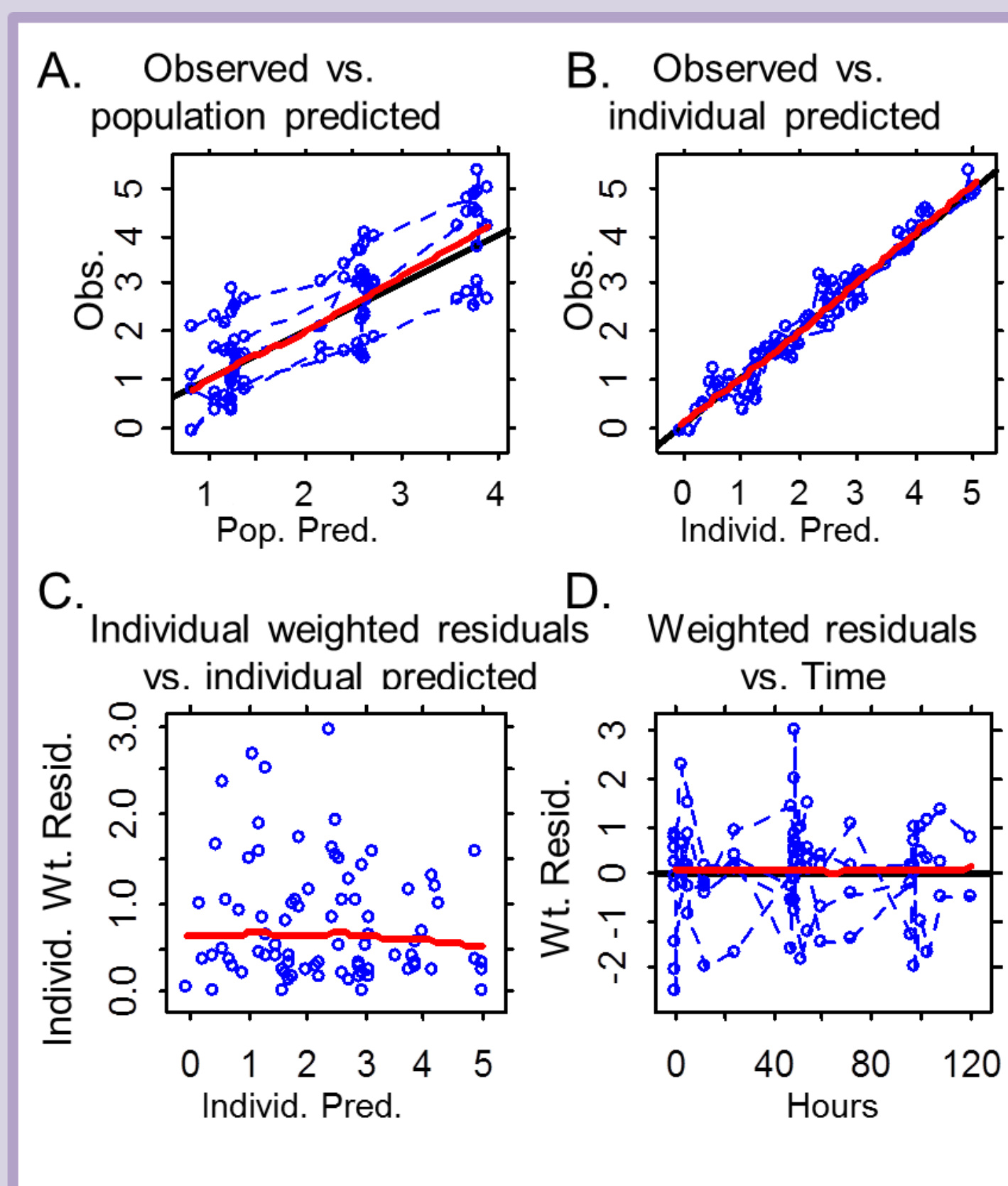


Figure 5. Comparison of observed versus predicted values for the population model is variable as expected but clearly shows a trend (A). Comparison of the individual fit models with data shows a reasonable fit and trend (B). Comparison of the weighted residuals and either predicted values or time show no trends (C and D).

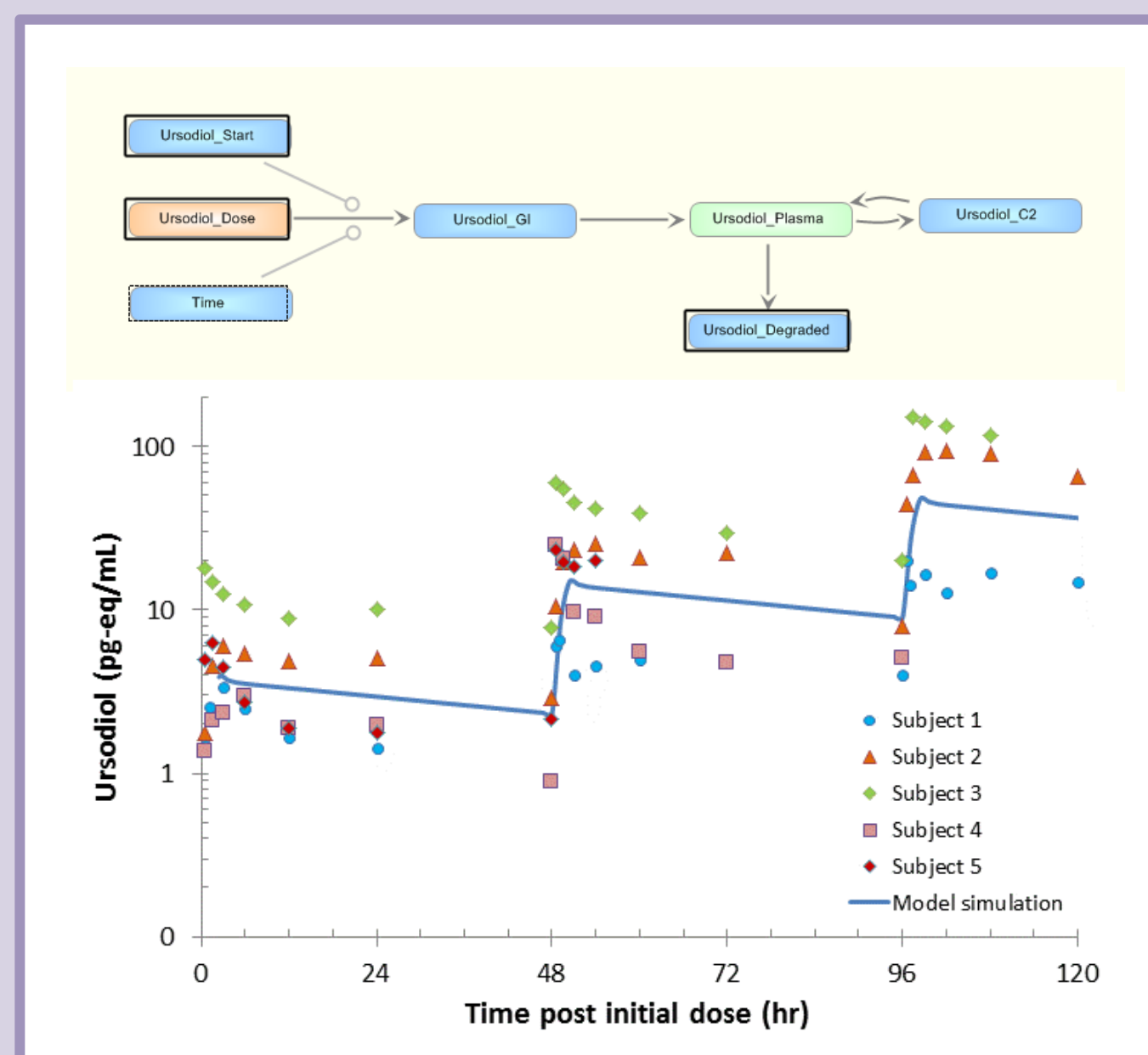


Figure 6. The 2 compartment population model was converted to a JDesigner ODE-based model and compared to the trial data. The blue line is the simulation results from the PK portion of the PhysioPD model plotted with the original data. As expected, the PK model provides a reasonable match with the data. This model was expanded and incorporated into the PhysioPD model of bile acid kinetics.

PK Conclusions

- A 2-compartment model fitted the data best.
- AMS can be used to study complicated PK in neonates.
- The sample volume and dose of labeled ursodiol can be lowered for any future studies and still provide measurable data.
- A physiological model of bile acid trafficking in adults had been developed and is being modified for infants.

PhysioPD Analysis

The PhysioPD modeling effort focused on developing a physiological model of bile acid metabolism which could be used to provide actionable insights into

- The variability in Ursodiol PK
- The biological effects of Ursodiol on bile acid metabolism (Ursodiol MOA)
- Incorporation of metabolomic data into a physiological model
- Translation of metabolism and PK between adult and pediatric models

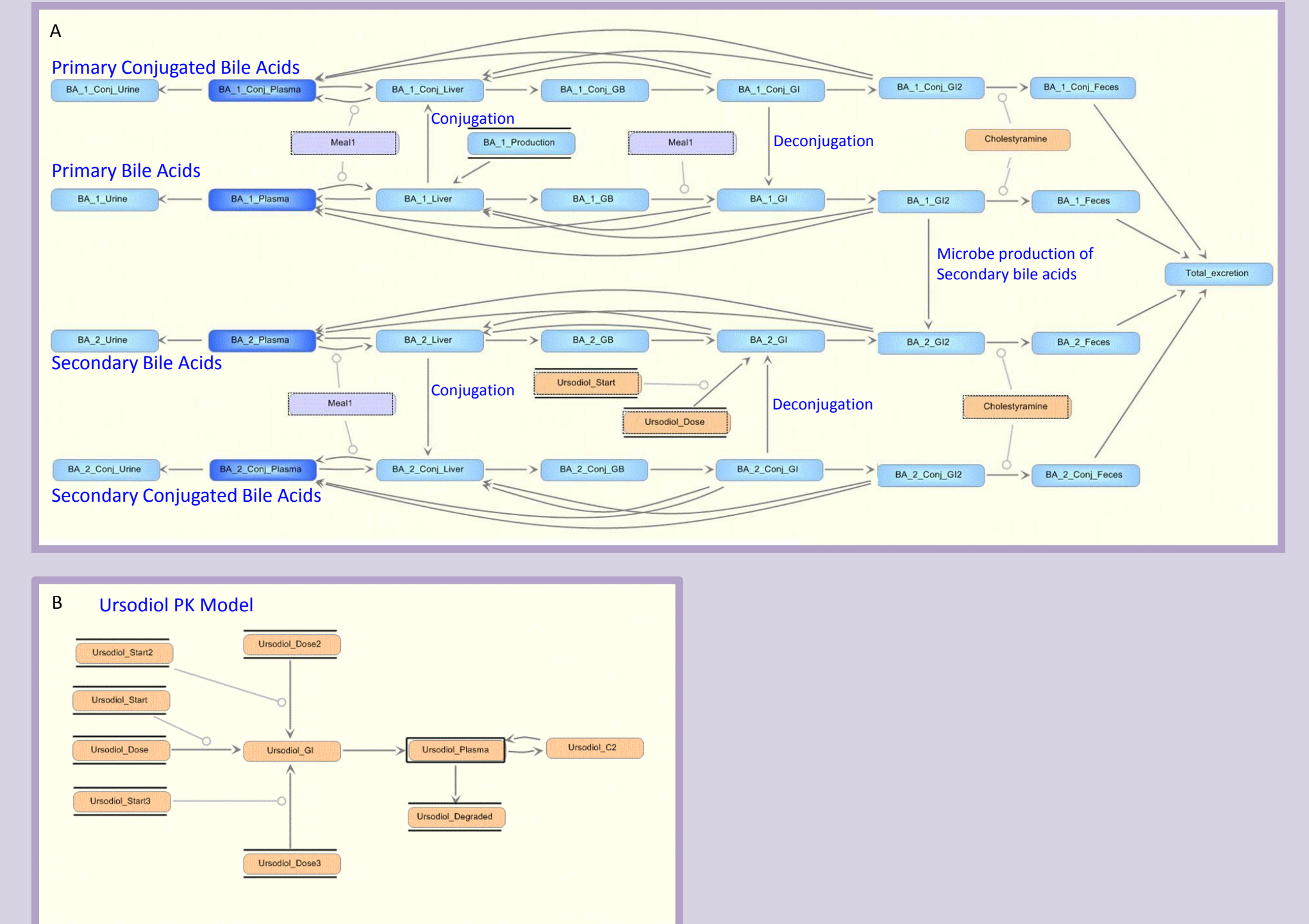


Figure 7. To evaluate the effects of ursodiol on bile acid kinetics, a PhysioPD model of adult bile acid metabolism has been developed. The model has a section of bile acid production, enterohepatic circulation and disposal (A). This section of the model incorporates data from literature as well as metabolomic data for plasma primary and secondary bile acids. In addition, the model contains a PK model of Ursodiol as derived in the PK section of this poster (B). The physiological section of the model did not use any of the PK data or information derived from the PK modeling work.

Figure 8. A secondary objective for this model was to test the incorporation and use of metabolomic data within a PhysioPD model. We chose to use a lipidomic analysis of bile acid metabolites which provided quantitative data for each of the primary and secondary bile acids, both unconjugated and conjugated. Figure 8 shows the cholesterol synthesis and bile acid metabolic pathways. Data from this lipidomic analysis was used for the plasma bile acid portion of the PhysioPD model.

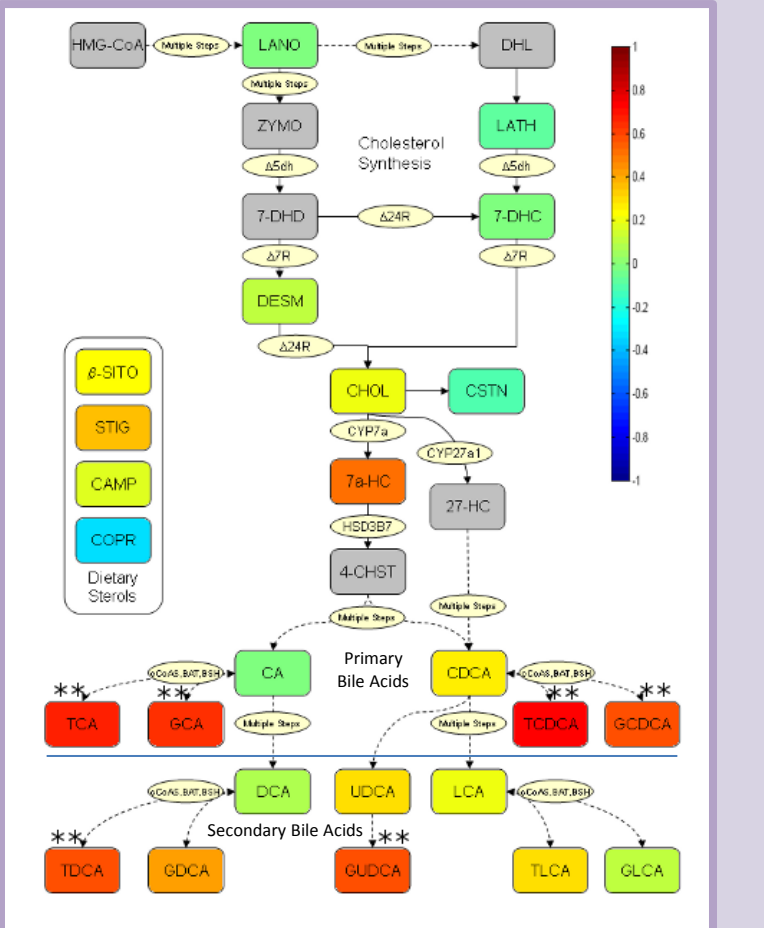


Figure from: Enteric Microbiome Metabolites Correlate with Response to Simvastatin Treatment. R. Kadivar-Dasik, K.A. Baillie, H. Zhu, Z.B. Zeng, M.M. Wiesl, J.T. Nguyen, K. Wajsborn, S.M. Watkins, M. Trupp, R.M. Krauss. Submitted to PLOS.

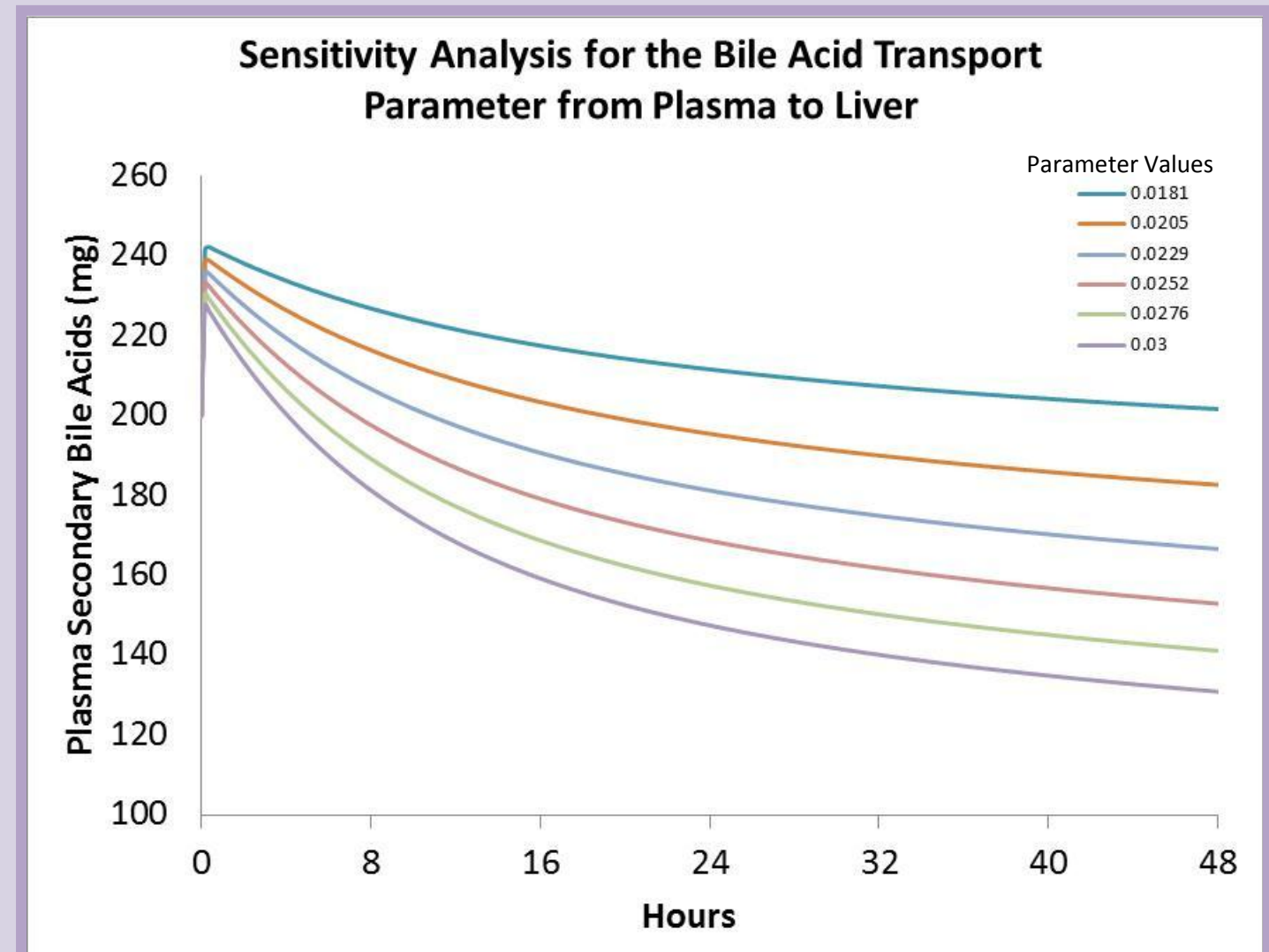


Figure 9. Sensitivity analysis for the parameter setting fasting secondary bile acid uptake from the plasma to the liver. The parameter was varied over a 10-fold range, a portion of which is shown here.

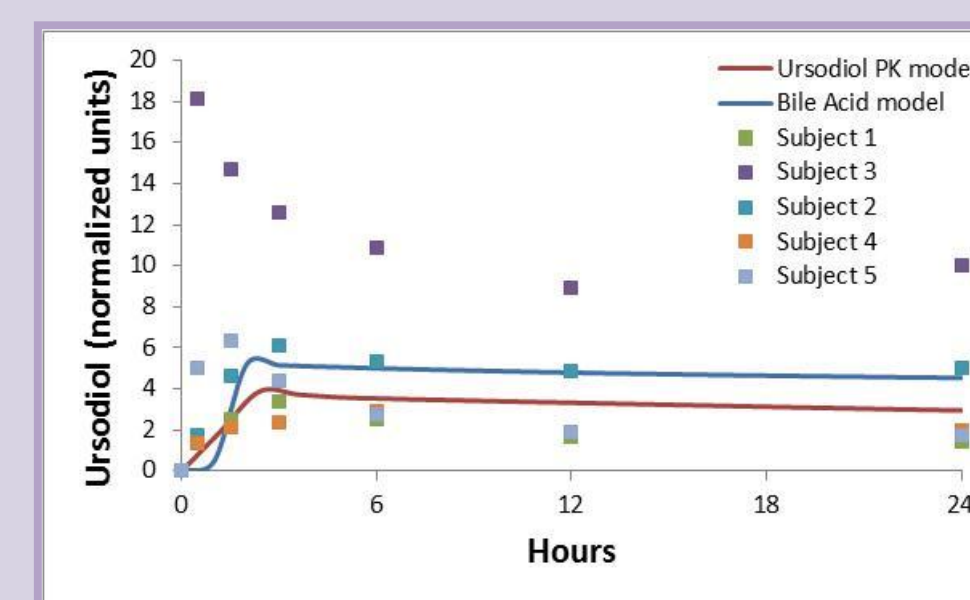


Figure 10. While some differences still remain, without tuning, the PhysioPD model can approximate the Ursodiol PK model. A simulation from the Ursodiol PK model (8 mg dose) is shown with patient data and the bile acid model output.

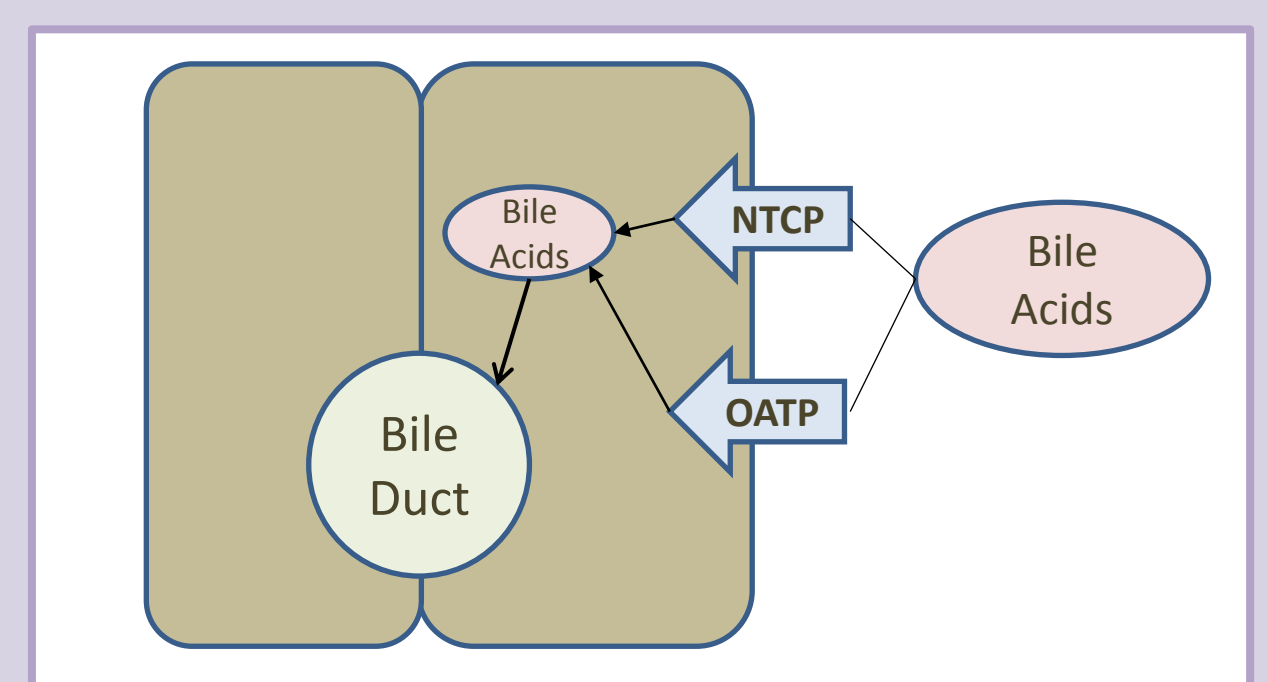


Figure 11. The most likely explanation for the variability in the C_{max} and terminal half-life of Ursodiol is polymorphisms in the organic anion transporter (OATP) and the sodium/bile acid cotransporter (NTCP)

PhysioPD Conclusions

- A physiological model of bile acid metabolism can be developed within a limited time frame to test and understand the variability and MOA of Ursodiol.
- Metabolomic data can easily be incorporated within PhysioPD models.
- Polymorphisms in the OATP and NTCP transporters are probable causes of Ursodiol PK variability.
- PhysioPD modeling indicates that Ursodiol PK will differ between fasted and fed subjects. The differences can be ascribed to a specific parameter within the physiological model which alters uptake of the drug into the liver.