Online Methods

The presented liver model is a multi-scale model comprising the metabolism of individual hepatocytes on cellular scale (Figure 1A), the individual sinusoidal unit on tissue scale (Figure 1B), the representation of lobulus via integration of multiple sinusoidal units (Figure 1C), the representation of the individual liver based on correlations between liver volume and blood flow and anthropomorphic features up to the variability in the population based on observed combination of anthropomorphic features in the population (Figure 1D).

Availability of data and models

All code and models and literature based datasets are made freely available. The cellular and sinuosoidal unit model are provided as SBML under creative commons (CC BY-SA 4.0) in the supplement and on Biomodels.org and JWS Online. A human-readable HTML representation of the model is provided in the supplement.

Numerical integration

The single hepatocyte and models of sinusoidal units are ordinary differential equation (ODE) based kinetic models. The models were integrated with libRoadRunner v1.3 {Somogyi2014, Somogyi2015} with absolute and relative tolerances of 1E-6. LibRoadRunner was further developed to efficiently handle very large SBML models via ...
All simulations and time courses were stored in a database.

Cellular scale - galactose metabolism

The kinetic model of galactose metabolism for individual hepatocytes consists of three main enzymatic steps i) the phosphorylation of galactose (gal) to galactose 1-phosphate (gal1p) catalysed by galactokinase (GALK, EC 2.7.1.6); ii) the conversion of gal1p to UDP-galactose (udpgal) by galactose-1-phosphate uridyl transferase (GALT, EC 2.7.7.10) and iii) the interconversion of udpgal and UDP-glucose (udpglc) by UDP-galactose 4´-epimerase (GALE, EC 5.1.3.2) {Novelli2000, Petry1998}. Galactose can enter glycolysis as glucose-1 phosphate (glc1p), one of the GALT reaction products, or can be incorporated as udpgal, the substrate donor of all galactosylation reactions, in glycoproteins and glycolipids {Novelli2000}. The alternative processes important in galactosemias and ATP synthesis (ATPS) and NADP reduction (NADPR) for cofactor regeneration were added to the model. Detailed information on metabolites, initial concentrations, rate equations and enzymatic parameters is provided in Supplementary Table 1 and Supplementary Table 2. The literature based kinetic parameters were included in SABIO-RK {Wittig2012} and annotated in the model (see Supplementary Tables and SBML annotations). Maximal enzyme activities (V_{max}) were chosen to achieve good correspondence of model simulations with reported galactose elimination rates in young subjects (20 years).

Sinusoidal Unit

The tissue-scale model of the sinusoidal unit (Figure 1B) consists of a central blood vessel (sinusoid) surrounded by the space of Disse and adjacent hepatocytes in cylindrical geometry

with parameters in Supplementary Table 3 and Supplementary Table 4. The periportal (pp) and perivenous (pv) blood compartments are located adjacent to the first and last sinusoidal volume, respectively. A single sinusoidal unit consists of N_c hepatocytes with each cell having a single associated sinusoid and Disse volume. In the sinusoid substances are transported by blood flow and diffusion, in the space of Disse solely by diffusion. Red blood cells (RBC) are constricted to the sinusoid, whereas all other model substances smaller than the fenestrae ($r_{substance} \leq r_{fen}$) pass in the space of Disse owing to the fenestration of the endothelial cells {Wisse1985}, i.e. galactose, albumin, sucrose and water. Galactose and water are exchanged between the space of Disse and the hepatocytes, whereas sucrose and albumin are restricted to the space of Disse.

Diffusion and blood flow are modelled via discretized one-dimensional diffusion and convection equations (analogue to {Konig2013}). The diffusion through the sinusoidal fenestration, small cylindrical channels in the endothelial cells is described via pore theory {Pappenheimer1953, Renkin1954}. The total restriction to diffusion due to the combined effects of steric hindrance at the entrance of the pores and frictional resistance within the pores for substance a with radius r_a is given as actual diffusion D_a relative to unhindered Diffusion $D_{a,0}$ with radius of the substance r_a and pore radius r_{fen} as

$$\frac{D_a}{D_{a,0}} = (1 - \frac{r_a}{r_{fen}})^2 \left[1 - 2.104(\frac{r_a}{r_{fen}}) + 2.09(\frac{r_a}{r_{fen}})^3 - 0.95(\frac{r_a}{r_{fen}})^5\right]$$ {Renkin1954}.

Heterogeneity of Sinusoidal Units

The heterogeneity of sinusoidal units within a lobulus was modeled via a Monte Carlo approach simulating a multitude of heterogeneous sinusoidal units based on experimental parameter distributions (Figure 2) for the the ultrastructure (sinusoidal length L_{sin}, sinusoidal radius y_{sin}, width space of Disse y_{dis}, hepatocyte sheet thickness y_{cell}) and microcirculation (sinusoidal blood flow velocity v_{blood}). The output of the lobulus is calculated as the integrated response over all sinusoidal unit samples in the region of interest (N_{sin}=1000). The parameter distributions were assumed log-normal and statistically independent of each other. Distributions of y_{sin}, v_{blood} and y_{cell} were fitted based on maximum-likelihood method for uni-variate distributions. For L_{sin} and y_{dis} the log-normal parameters were calculated from reported mean m and standard deviation std. The resulting distribution parameters and experimental data are given in Supplementary Table 4.

Variation in perfusion is modeled by scaling the distribution of sinusoidal blood flows $p(v_{blood})$ via $p_{f=f_{flow}}(v_{blood}) = p(f_{flow} v_{blood})$ to higher or lower blood flows with $p_{f=1}$ corresponding to the experimental microcirculation.

Integration of Sinusoidal Units (Lobulus)

To calculate the response of a lobulus the simulation results of N_{sin} sinusoidal units under identical periportal boundary conditions are integrated, each sampled from the parameter distributions corresponding to the simulated conditions. For instance the lobulus perfusion is

calculated from the volumes V[k] and blood flows Q[k] for individual sinusoidal with $x_{tot} = \sum_{k=1}^{N_{sin}} x[k]$ and $< x > = \frac{1}{N_{sin}} \sum_{k=1}^{N_{sin}} x[k]$ as $P_{sin} = \frac{Q_{sintot}}{V_{sintot}} = \frac{\sum_{k=1}^{N_{sin}} Q_{sin}[k]}{\sum_{k=1}^{N_{sin}} V_{sin}[k]}$

This integration over the sinusoidal units only accounts for the parenchymal fraction of the liver volume of around 80% (f_{tissue}=0.8). Accounting for the non parenchymal volume of the liver, consisting mainly of large vessel volume the tiss volume V_{tissue} is calculated from the sinusoidal liver volume V_{sin} as

$V_{tissue} = V_{sin} + V_{ves} = (2 - f_{tissue}) V_{sin}$

resulting in the tissue perfusion

$P_{tissue} = \frac{Q_{sintot}}{V_{tissue}} = \frac{1}{(2-f_{tissue})} \frac{Q_{sintot}}{V_{sintot}}$

The integration of tissue galactose elimination and clearance is performed in an analogue way.

Multiple Indicator Dilution Curves

The multiple indicator dilution curves under varying unlabeled galactose concentration were modeled via: i) running simulation to steady state under given unlabeled galactose concentration; ii) giving a periportal tracer peak of duration 0.5s. The hepatic vein tracer concentration for substance s is calculated as flow weighted average of the perivenous time courses of the individual sinusoidal units $c_{pv}^{s}[k]$, i.e.

$$c_{ven}^{s}(t) = \sum_{k=1}^{N_{sin}} w_k c_{pv}^{s}[k](t) = \sum_{k=1}^{N_{sin}} \frac{Q_{sin}[k]}{Q_{sintot}} c_{pv}^{s}[k](t)$$

For the comparison with experimental data the catheter and nonexchangeable vessel transit time t_0 were estimated from the time of first appearance of radioactivity above background levels in the experimental and simulated dilution curves. This zero point was used for mapping simulations and experiments. The dilution curves are simulated with reported GEC values for dogs of ~ 0.5 * GEC of humans (see supplement).

Galactose Elimination, Extraction Ratio and Clearance

The galactose elimination rate (GE), extraction ratio (ER) and clearance (CL) for a single sinusoidal unit k are calculated from sinusoidal blood flow Q_{sin}[k] and periportal and perivenous galactose concentrations $c_{pp}^{gal}[k]$ and $c_{pv}^{gal}[k]$ in steady state {Schirmer1986}

$$GE[k] = Q_{sin}[k](c_{pp}^{gal}[k] - c_{pv}^{gal}[k])$$

$$ER[k] = \frac{c_{pp}^{gal}[k] - c_{pv}^{gal}[k]}{c_{pp}^{gal}[k]}$$

$$CL[k] = Q_{sin}[k] \frac{c_{pp}^{gal}[k] - c_{pv}^{gal}[k]}{c_{pp}^{gal}[k]} = Q_{sin}[k]\, ER[k]$$

The integrated GE, ER and CL per tissue volume liver for N_{sin} sinusoidal units, are calculated with the volume of the individual sinusoidal units V[k] as

$$GE_{tissue} = \frac{1}{(2-f_{tissue})} \frac{Q_{sintot}}{V_{sintot}} < c_{pp}^{gal}[k] - c_{pv}^{gal}[k] >= P_{tissue} < c_{pp}^{gal}[k] - c_{pv}^{gal}[k] >$$

$$ER_{tissue} =< \frac{c_{pp}^{gal}[k] - c_{pv}^{gal}[k]}{c_{pp}^{gal}[k]} >$$

$$CL_{tissue} = \frac{1}{(2-f_{tissue})} \frac{Q_{sintot}}{V_{sintot}} < \frac{c_{pp}^{gal}[k] - c_{pv}^{gal}[k]}{c_{pp}^{gal}[k]} >= P_{tissue} < \frac{c_{pp}^{gal}[k] - c_{pv}^{gal}[k]}{c_{pp}^{gal}[k]} >$$

Clearance based on equilibrium galactose concentrations overestimate hepatic clearance of galactose especially at very low galactose concentration due to small basal systemic galactose clearance R_{base} outside kidney and liver as reported by {Keiding1988} and discussed in {Waldstein1960}. Consequently, the experimental data for ER and CL {Tygstrup1958, Tygstrup1954, Waldstein1960, Henderson1982, Winkler1965, Palu1965} was corrected for R_{base} with V^R_{max} =0.114 [mmol/min] fitted with the data from {Keiding1988} and K^R_m = 0.2 mM in the range of galactokinase Km for galactose. The correction calculates depending on the equilibrium galactose concentration the respective systemic basal clearance

$$R_{base}(gal_{eq}) = V^R_{max}(\frac{gal_{eq}}{gal_{eq} + K^R_m})$$

giving corrected experimental clearance and galactose elimination as

$$GE_{liver} = GE_{exp} - R_{base}$$

and

$$CL_{liver} = CL_{exp} - \frac{R_{base}}{gal_{eq}}$$

The estimated maximal systemic GEC is ~4% of the hepatic GEC.

Alterations in aging

Changes in ultrastructure of the liver (pseudocapillarization) were modeled via decreasing the parameters for fenestration number per area (N_{fen}), and increasing the endothelial thickness (y_{end}) with age based on experimental data. Simulations were performed with three parameter sets corresponding to 20 years, 60 years and 100 years (interpolated) (supplementary information). The GE response curves were interpolated for the ages in between.

Scaling to the liver - Individualized predictions

Based on the integration of detailed kinetic models of sinusoidal units over a region of interest the metabolic function for given liver structure, morphology, perfusion and metabolic function can be calculated.

The total hepatic function of a person results from this regional function with the actual liver volume and blood flow of the person. This is achieved via prediction of the individual liver characteristics based on the anthropomorphic data of the person, i.e. based on sex, age, bodyweight, height and body surface area.

In a first step generalized additive models for location, scale and shape (GAMLSS) {Stasinopoulos2007} were fitted to describe the correlations between single liver features and single anthropomorphic features based on individual data from >3000 subjects from >30 studies (supplement GAMLSS). Individual models for male, female and all data were fitted. The resulting models enable the prediction of the probability distributions of liver features for single anthropomorphic features based on gender, for instance the distribution of liver volumes depending on age for femalespsex=F,volLiverage.Importantly, the observed population variability is part of the model.

The age dependent change in total liver volume, blood flow and perfusion are taken into account in the age-dependent GAMLSS curves.

In a second step, the information of the single feature models is combined under the assumption of statstical independence to generate a best estimate of liver volume based on the set of antropomorphic features observed in the person. The result is a personalized probability distribution of liver volumes p_k(volLiver) for the subject k with sex=S, age=A, bodyweight=B, height=H and bsa=BS

pk(volLiver)= p(volLiver|sex=S, age=A, bodyweight=B, height=H, bsa=BS)=
psex=S,volLiverage(volLiver|age=A)
psex=S,volLiverkgage(volLiver|age=A, bodyweight=B)
psex=S,volLiverbodyweight(volLiver|bodyweight=B)
psex=S,volLiverkgbodyweight(volLiver|bodyweight=B)
psex=S,volLiverheight(volLiver|height=H)
psex=S,volLiverkgheight(volLiver|height=H, bodyweight=B)
psex=S,volLiverbsa(volLiver|bsa=BS)
psex=S,volLiverkgbsa(volLiver|bsa=BS, bodyweight=B)

Hepatic blood flows is calculated in a similar manner, but taking the additional correlation information between liver volume and blood flow into account. The resulting probability distribution of hepatic blood flow for person k with given liver volume V is

pk(flowLiver|volLiver=V)=p(flowLiver|sex=S, age=A, bodyweight=B, bsa=BS)psex=S,flowLivervolLiver(flowLiver|volLiver=V)
psex=S,flowLiverage(flowLiver|age=A)
psex=S,flowLiverkgage(flowLiver|age=A, bodyweight=B)
psex=S,flowLiverbodyweight(flowLiver|bodyweight=B)
psex=S,flowLiverkgbodyweight(flowLiver|bodyweight=B)
psex=S,flowLiverbsa(flowLiver|bsa=BS)
psex=S,flowLiverkgbsa(flowLiver|bsa=BS, bodyweight=B)
psex=S,flowLivervolLiver(flowLiver|volLiver=V)

Finally, the metabolic function of person k, in this case the galactose elimination capacity, is calculated by scaling the metabolic function per tissue volume for given perfusion and structure, to total blood flow and liver volume ($flowLiver_k$ and $volLiver_k$) via the metabolic functions calculated for regions of the liver.

$GEC_k = f_{GEC\_per\_volLiver}(flowLiver_k/volLiver_k) * volLiver_k$

Via Monte Carlo simulation, i.e. repeated sampling from the individualized probability distributions pk(volLiver) and pk(flowLiver|volLiver)the distribution of liver volumes, blood flows and metabolic function in people with the given anthropomorphic features can be calculated.

Body surface was (BSA) is calculated via DuBois formula from bodyweight and height BSA=0.007184bw0.725h0.425{Moesteller1987} .

Population variability

To calculate the population variability in liver function the prediction of liver volume, blood flow and GEC was performed for a large cohort representative of the US population. The NHANES {NHANES} survey data between years 1999 - 2012 was used, with subjects filtered based on body mass index ($18.5 \leq BMI \leq 24.9$) and ethnicity (Non-Hispanic White). For all subjects with complete data sets of age, gender, height, and body weight the prediction was performed. Using the Monte Carlo approach, repeated computations based on inputs selected at random from statistical distributions for each input parameter are conducted to provide a statistical distribution of the output. Using high percentile (e.g. 95th) and 50th percentile, the

intraspecies variability can be calculated. To derive this information, Monte Carlo simulations based on distributions of input parameters have frequently be used. (Lipscomb et al., 2003; Gentry et al., 2002; Haber et al., 2002; Lipscomb and Kedderis, 2002; Timchalk et al., 2002; Bogaards et al., 2001; El-Masri et al., 1999; Thomas et al., 1996a, b).

Classification & ROC curves

ROC curves were calculated based on the following classificator
TODO

Performance of the classifier was evaluated against logistic regression on the same datasets.