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}.

Convection

$$v^{conv}_{Si \rightarrow Sj}(k) = Q_{Si} \cdot c_{Si}(k)$$

Filtration & reabsorption

$$v^{conv}_{Si \rightarrow Di}(k) = q_{Si} \cdot \Delta x \cdot c_{Si}(k)$$

Diffusion in sinusoid

$$v^{diff}_{Si \rightarrow Sj}(k) = \frac{D_{k,0}}{\Delta x \cdot A_{sin}} \cdot (c_{Si}(k) - c_{Sj}(k))$$

Diffusion sinusoid & space of Disse

$$v^{diff}_{Si \rightarrow Di}(k) = \frac{D_{k,a}}{\Delta y \cdot A_{sin-disse} \cdot f_{fen}} \cdot (c_{Si}(k) - c_{Di}(k))$$

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

Diffusion space of Disse

$$v^{diff}_{Di \rightarrow Dj}(k) = \frac{D_{k,0}}{\Delta x \cdot A_{disse}} \cdot (c_{Di}(k) - c_{Dj}(k))$$

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 $c^s_{pv}[k]$, i.e.

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

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_{max}^{R} =0.114 [mmol/min] fitted with the data from {Keiding1988} and K_m^R = 0.2 mM in the range of galactokinase Km for galactose. The correction calculates depending on the equilibrium galactose concentration gal_{eq} the respective systemic basal clearance

$$R_{base}(gal_{eq}) = V_{max}^{R}(\frac{gal_{eq}}{gal_{eq} + K_m^R})$$

giving corrected experimental clearance and galactose elimination as

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

and

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

Extrahepatic, intracorporeal removal of galactose is likely. In animals which have undergone hepatectomy, nephrectomy and evisceration, galactose disappears from the blood although at a slow rate [17]. Studies in which radioactive C14 galactose has been used have demonstrated metabolism of galactose by tissues other than the liver [18]. One explanation => is small but constant amount of extrahepatic removal. The estimated maximal systemic GEC is ~4% of the hepatic GEC.

For comparison with the experimental data the per tissue model predictions were scaled to the complete liver under assumption of V_{liver}=1500ml resulting in blood flow $Q_{liver} = P_{tissue}V_{liver}$, galactose elimination $GE_{liver} = GE_{tissue}V_{liver}$ and clearance $CL_{liver} = CL_{tissue}V_{liver}$.

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.

Individualized GEC predictions

Personalized GEC is calculated by combining a predictor for liver volumes and blood flow from anthropomorphic information (age, body weight, height, BSA) with the multiscale model galactose elimination curves (GE), allowing the calculation of age-dependent GEC for given perfusion in volume of liver tissue.

The dependencies between liver volume/blood flow and anthropomorphic features are described via generalized additive models for location, scale and shape (GAMLSS) {Stasinopoulos2007} fitted to individual data from >3000 subjects from >30 studies (supplement GAMLSS). The resulting models enable the prediction of probability distributions of liver features for given anthropomorphic features, e.g. the distribution of liver volumes depending on age, body weight or height. In a second step, the prediction of liver volume for a set of anthropomorphic features is generated by combining the single feature models (assumption of statistical independence). 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. Hepatic blood flows is calculated analogue, but integrates the additional correlation between liver volume and blood flow, resulting in a distribution of liver blood flows for given liver volumes and anthropomorphic features p_k(flowLiver|volLiver).

GEC for person k from total blood flow ($flowLiver_k$) and liver volume ($volLiver_k$) results as

$GEC_k = f_{GEC\_per\_volLiver,\ age=agek}(flowLiver_k/volLiver_k, gal=8mM) * volLiver_k$

Via repeated sampling from the individualized probability distributions pk(volLiver) and pk(flowLiver|volLiver) the distribution of liver volumes, blood flows and corresponding GEC is calculated.

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

The multiscale model classification of disease status is based on the standard deviation sd_{GEC} and the 5% quantile q_{GEC} of the distribution of predicted GEC values for given anthropomorphic features and the experimental GEC value

$$p_{multiscale} = \frac{q_{GEC} - GEC}{sd_{GEC}}$$

Performance of the classifier was evaluated against logistic regression with various predictors on identical datasets (corresponding to the maximal dataset containing values for all predictor variables necessary for the respective logistic regression model). Logistic regression performance was calculated via bootstrap model fitting (N_B=100). Fitting of logistic models was implemeted in R. AUC and ROC curves were calculated with R package ROCR. The multiscale model classifier was implemented as a web application in Shiny R.