A Multiscale Computational Model Predicts Human Liver Function From Single-Cell Metabolism

König M.[1], Marchesini G.[2], Vilstrup H.[3], Somogyi A.[4] and Holzhütter HG.[1]

[1]Department of Computational Systems Biochemistry, University Medicine Charité Berlin, D-10117 Berlin, Germany
[2]Department of Internal Medicine, Aging and Nephrological Diseases, University of Bologna, I-40138 Bologna, Italy
[3]Department of Hepatology and Gastroenterology, Aarhus University Hospital, DK-8000 Aarhus, Denmark
[4]Biocomplexity Institute, Indiana University, Simon Hall MSB1, Bloomingtom, IN 47405

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To whom correspondence should be addressed: Matthias König, Institute of Biochemistry, University Medicine Charité Berlin, Virchowweg 6, 10117 Berlin; Tel: (0049) 30450528197; Email: matthias.koenig@charite.de

ABSTRACT

Understanding how liver function arises from the complex interaction of morphology, perfusion, and metabolism from single cells up to the entire organ requires systems-levels computational approaches. We report a multiscale mathematical model of the Human liver comprising the scales from single hepatocytes, over representation of ultra-structure and micro-circulation in the hepatic tissue, up to the entire organ integrated with perfusion. The model was validated against data on multiple spatial and temporal scales. Herein we describe the model construction and application to hepatic galactose metabolism demonstrating its utility via i) the personalization of liver function tests based on galactose elimination capacity (GEC), ii) the explanation of changes in liver function with aging, and iii) the prediction of population variability in liver function based on variability in liver volume and perfusion. We conclude that physiology- and morphology-based multiscale models can improve the evaluation of individual liver function.

INTRODUCTION

The liver is the metabolic center of our body performing hundreds of functions including the homeostasis of numerous plasma metabolites; producing bile; detoxification of xenobiotics; and clearance of drugs and substances like galactose. In the past 30 years enormous progress in the knowledge and management of liver disease has been achieved, yet approximately 29 million people in the European Union still suffer from a chronic liver condition with underlying mechanism often being unclear {Blachier2013}. Liver function is the result of complex interplay of hepatic morphology, perfusion and metabolism across multiple spatial domains, from the cellular level up to the entire organ {Rappaport1979}. Computational models are uniquely positioned for the analysis of such complex systems and to capture the connectivity between these divergent scales and.

Liver Architecture

Liver architecture is unique in that it consists of a multitude of microscopic functional units termed lobules, which are connected in parallel to the blood flow. Within a single lobule a network of capillaries, the liver sinusoids transport the blood from the outer periportal regions, supplied via the portal vein and hepatic artery, to the central perivenous region, drained by the central vein {Sasse1992, Rappaport1979, Rappaport1973}. The sinusoidal unit, a single capillary perfused with blood and lined by a layer of hepatocytes forms the smallest functional unit of the liver (Figure 1) {Bass1977}. The fenestrated endothelial cells of the liver sinusoids act as a dynamic filter that permits exchange of fluid, solutes and particles between the sinusoidal lumen and space of Disse adjacent to the hepatocytes {Cogger2003}.

GEC as Liver function test

Quantification of liver function is necessary to assess the degree of liver impairment, to evaluate response to treatment and to select transplant recipients {Dufour1992}. Most function tests are based on the rate with which a given test substance is cleared specifically by the liver. Particularly, the liver is the primary organ for clearance and whole-body metabolism of galactose {Bernstein1960, Berry2000, Segal1971}. The determination of the maximal galactose removal rate, the galactose elimination capacity (GEC) {Marchesini1988, Schirmer1986, Tygstrup1966}, which is an established test of liver function measured in numerous studies {Jepsen2009, Fabbri1996}. Impairment of the liver in diseases like cirrhosis {Henderson1982, Jepsen2009} are associated with reduced GEC. Preoperative GEC predicts complications and survival after hepatic resection {Redaeli2002} and as predictor of survival in cirrhosis {Merkel1991, Salerno1996}.

Alterations in Aging

The percentage of deaths attributed to liver disease increases dramatically in humans beyond the age of 45 years {Schmucker2005}. In the elderly, a marked reduction in quantitative liver function measured by GEC {Schnegg1986, Marchesini1988} as well as major physiologic changes affecting liver function, i.e. decline in liver volume and blood flow {Anantharaju2002, Wynne1989, Marchesini1988}, are observed. In addition, characteristic changes in ultrastructure

termed pseudocapillarization occur with aging, characterized by defenestration, thickening of the endothelium, and deposition of basal lamina and extracellular matrix in the space Disse {McLean2003, LeCouteur2001 , Cooger2003}. Age-related changes in the liver sinusoids are implicated in the association between ageing and impaired clearance of drugs {LeCouteur1998} and may provide a mechanistic link between primary aging processes and age-related disease {LeCouteur2002, Cogger2003}. Surprisingly, it is not clear to which extent age-inherent alterations in microangio-architecture, microvascular haemodynamics and liver volume and perfusion are contributing factors of age-related susceptibility of the liver {Vollmar2002}.

Multiscale-Model

Systems-level computational approaches are required to elucidate the complex interaction of organ structure, perfusion, and metabolism on multiple scales and to understand how these influence liver function, here the clearance of galactose and GEC. They are uniquely positioned to capture the connectivity between these divergent scales, as they can bridge the gap in understanding between isolated in vitro experiments and whole-organ in vivo models {Walpole2013}. Our objective was to develop such a multiscale computational model which i) describes physiology, morphology and function of the human liver; ii) can be applied to the evaluation of liver function tests, i.e. GEC; iii) can predict the effects of altered perfusion, liver volume and ultrastructure like occurring in aging on liver function; iv) can be integrated with patient data, and v) has clinical relevance.

RESULTS

We present a multiscale mathematical model of the human liver bridging the scales from individual cellular processes to the level of the entire organ. The model describes physiology, morphology and function of the human liver by integrating hepatic galactose metabolism with perfusion and liver structure (Figure 1). The model integrates and predicts experimental data on multiple temporal and spatial scales: i) prediction of hepatic multiple indicator dilution curves (Figure 2); iii) prediction of heterogeneity within sinusoids (hepatic zonation) and between sinusoids (Figure 2 and 3); prediction of galactose extraction, clearance and extraction fraction (Figure 4); iii) prediction of individual GEC, population variability in GEC and alterations in GEC with aging (Figure 4). We developed a classifier for liver disease based on our personalized model predictions for GEC outperforming regression approaches in a retrospective analysis of a large cohort study (Figure 4). We demonstrate possible clinical application of the presented systems biology approach by implementation of the classifier into a web application for simple use (Figure 5).

Multiscale Model of Human Liver

Our approach was … (independent units which could be tested, i.e. self consistent cell model,.. integration of scales & approaches)

Hepatocyte - Our model combines detailed kinetic models of cellular metabolism (Figure 1A) with a tissue-scale perfusion model of the sinusoid (Figure 1B). Despite the importance of the hepatic galactose metabolism for the systemic galactose clearance and formation of precursors

for glycosylation reactions no detailed mathematical model of this pathway is available hitherto. This work presents to our knowledge the first kinetic model of galactose metabolism in hepatocytes (Figure 1A) comprising among others the three key enzymatic steps of galactose metabolization: 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}.

Sinusoidal Unit - The cellular model is integrated into a realistic tissue-scale model of the sinusoidal liver unit based on known histological parameters (geometry, cell numbers, architecture, perfusion rates, …) (Figure 1C). Important features of liver architecture important for elimination, namely fenstraeted endothelial cells and space of Disse are explicitely represented in the model (see Methods).

Lobulus - Regional liver metabolism was modelled as weighted average across the heterogeneous contributions of tissue-scale models with differing in blood flow rates and tissue structure (Figure 1D, Figure 2A for parameter distributions). Similar to the classical distributed models of liver elimination {Bass1978}, but with an explicit description of ultrastructure and detailed kinetic models in the hepatocytes.

Mean sinusoidal unit & the integrated response over the heterogeneous contributions of sinusoids based on heterogeneity in ultra-structure and microcirculation are presented.

Liver anatomy ensures that periportal concentrations are common in all sinusoidal units. Outflow concentrations cv are assumed to be well mixed when they reach the hepatic vein.

Organ - Finally, the function of the entire organ is modelled by scaling of the regional output based on individual liver perfusion and liver volume, resulting in individualized liver function.

Personalization - Personalized multiscale models are generated based on individual anthropomorphic information in combination with fitted relationships describing the dependencies of hepatic volume and blood flow on these features.

Multiple Dilution-Indicator Curves

In a first step the model was validated multiple-indicator dilution curves (Figure 2BC) {Goresky1973}. The single-injection, multiple-indicator dilution approach provides a method to determine the composition of the liver and the rates of hepatic processes {Goresky1973}. Labeled red blood cells (RBC) are used as vascular reference. Larger materials are excluded from the space of Disse. The outflow concentration of each tracer is divided by the total injected, providing a normalized value, an outflow fraction per ml. The integrated behavior based on molecular detailed description of single cell behavior give the correct multiple dilution indicator curves, consequently describing correctly the distribution of substances in the various spaces.

At low blood galactose concentrations, the labeled galactose appears at the outflow with labeled sucose, but is much reduced in magnitude, and exhibits a long tailing. Its outflow recovery is much reduced. At high blood galactose concentrations, the initial part of the profile increases

towards that for labeled sucrose, the tailing becomes much larger in magnitude, and the outflow recovery becomes virtually complete {Goresky1973}. The shift of the dilution-indicator curves observed and explained by Goresky as different free volumes of the substances are a consequence of the different diffusion coefficients for the substances, and consequently altered distribution kinetics within the sinusoid and space of Disse resulting in a delayed appearance of the substances perivenous. None of the data was used for model fitting, all model parameters result from the geometric constraints of the system and the physiochemical properties of the substances transported within the sinusoid and the space of Disse. Only the exchange rates between hepatocytes and the space of Disse could be adapted, in the case of water freely, in case of galactose these fluxes are constrained by the total galactose elimination per volume tissue.

Heterogeneity between Sinusoids and within sinusoids

Our multiscale-model allows predictions about the cell to cell variability and the heterogeneity within the lobulus, i.e. between different sinusoidal units. By explicitly accounting for the observed heterogeneity in sinusoidal blood flow and ultrastructure (Figure 2A) we could analyze the local heterogeneity between different sinusoidal units in the tissue (Figure 2D). Due to the detailled modeling of the individual hepatocytes along the sinusoid the zonation patterns and gradients from periportal to perivenous could be analysed (Figure 3).
Individual cells within the sinusoid as well as different sinusoid show large differences in their time courses and local concentrations. Depending on local flow and morphology as well as location along the periportal-perivenous axis the acual concentration profiles are very heterogenous under identical periportal input concentrations.
Discuss, implications (Could explain the observed heterogeneity observed in NAFDL, locally differnent concentrations, ...).

Hepatic Galactose Elimination

Central to understanding the physiology of organ function and pathophysiology of organ dysfunction is the awareness of organ perfusion {Schirmer1986}.
The extent to which flow or metabolic function determines the rate of clearance depends on the biochemical efficiency of the liver for removal of the substrate relative to flow {Schirmer1986}.

Via integration of the heterogenous sinusoidal units the hepatic galactose clearance, extraction ratio (ER) and the galactose elimination capacity for given perfusion in a liver volume can be calculated (see methods). The model reproduces the observed saturation in galactose clearance, the dependency of the extraction ration of galactose on the perfusion (Figure 4AB).
Hepatic galactose elimination follows Michaelis-Menten saturation kinetics {Keiding1973, Keiding1976} with a concentration-dependent (first-order) elimination phase at low galactose concentrations and a definable clearance maximum, the galactose elimination capacity (GEC), at higher concentrations (zero-order phase) {Schirmer1986}. Important determinants in galactose clearance are hepatocyte function and liver blood flow, with low galactose clearance measuring the estimated hepatic blood flow, whereas high galactose concentration measures the hepatic functional capacity (Figure 4C).

[Figure 4 will be discussed in the context of enzyme limited & flow limited below]

Three hepatic clearance regimes (flow-limited, general and enzyme-limited) can be defined from a model of hepatic perfusion-elimination relationships {Winkler1978}

High galactose (GEC) - The enzyme limited clearance regime

The functional capacity can be evaluated by measuring the rate of elimination at a sufficiently high concentration, where the eliminatory mechanisms are saturated {Winkler1978}.

Under high galactose concentrations like occuring in the galactose clearance tests the model predicts a capacity limited galactose clearance, thereby measuring the galactose elimination capacity. Hepatic vein catheterization at high concentrations revealed a constant hepatic arterial-hepatic venous concentration difference {Tygstrup1954, Tygstrup1958}. Since all hepatocytes are thought to participate maximally in the removal of galactose, the test has been regarded as a measure of the functioning liver cell mass (Lm) {Tygstrup1966}.

The clearance does not vary with the perfusion and is proportional to Vmax (substances with Vmax/FKm $\ll 1$) {Winkler1979, Schirmer1986}

Low galactose - The flow limited clearance regime Galactose Clearance at low concentrations has proven to be as near ideal a method for estimating the effective hepatic blood flow (EBHF) because most of the plasma galactose entering the liver also leaves the liver without being metabolized {Schirmer1986}. Henderson et al. confirmed the near complete extraction of galactose across the liver (ER = 0.94) in subjects without hepatic disease by performing hepatic vein catheterization {Henderson1982}. Similar results were obtained by Keiding et al. with ER = 0.91±3 {Keiding1988}.

The most extensive work on galactose elimination kinetics was done by Keiding and co-workers {} [43-45, 48, 50, 85, 99]. “These kinetic studies on the clearance of galactose at concentrations of 0 to 10 mg/dl (0 to 0.555 mmol/l) show that it approaches the ideal test substance for measuring effective liver blood flow (EHBF) (a) it is kinetically simple to analyse at steady state during continuous infusion (b) it is avidly removed by the liver, with minimal extrahepatic clearance and c) there is indirect evidence supporting virtually complete extraction by functional liver tissue on each pass.

When a substance is removed in the liver by a process with a large amount of enzyme and a high affinity (low Km) relative to the hepatic blood flow (Vmax/FKm $\gg$ 1) the removal is completely determined by the perfusion (all substance removed in a single passage). This flow-limited regime can be used for the measurement of the perfusion.

It is not always possible to design tests of liver functions based on a measurement of Vmax at high blood concentration in man, because these may be toxic or have unwanted haemodynamic or osmotic effects or because the large amount of test substances may be expensive {Winkler1978}. A model which provides means to calculate the complex interactions between perfusion and metabolism in the various elimination regimes can evaluate also the cases where flow has a strong effect on clearance

Galactose Elimination

Waldstein demonstrated that the extra-renal elimination of galactose from the body would reach a maximum during galactose infusions {Waldstein1960}.

Galactose Clearance

Galactose Extraction Ratio

Arterio-hepatic venous concentration difference

Liver-vein catheterization studies have shown that the arterio-hepatic venous concentration difference is constant in a wide concentration interval [Tygstrup & Winkler 1954].

Metabolic effects

“In animals with saturated meatbolism (i.e. blood galactose concentrations > 2mmol/l), the liver concentrations are significantly increased in relation to the control animals for galactose-1-p, and UDP-galactose, and a significant reduction is seen for UDP-glucose, ATP and the sum of adenine nucleotides {Keiding1973, rat}.

Personalized GEC prediction

Reference ranges play an important role in clinical medicine, with values that lie outside the reference range viewed as an indication for further investigation and/or treatment {Cole2009}.

“The physiological variation in GEC (refs, Figure …) implies that it may be impossible to decide if the liver function is normal or not from a single determination of GEC, but if a reference value is obtainable, either in the same subject or in a comparable group, small variations in the liver function may be detectable {Tygstrup1964}“

The estimation is based on predicted liver volumes and blood flows.
The liver volume is the deterimening factor, but for other drugs / eliminated compounds the flow could be important (depending on the clearance regime of the substance.
‘The significant correlation of GEC to BSA may indicate that the elimination capacity depends on the size of the liver (liver mass, Lm) {Tygstrup1964}

The regional galactose elimination curves giving the integrated galactose elimination within a lobulus/region of interest of the liver based on the occuring heterogeneity for given perfusion in the region can be scaled to the total liver by scaling the response of a region to total liver volume and perfusion. We developed a method of estimating individual hepatic blood flow and liver volume from anthropomorphic information, i.e. age, gender, bodyweight, height and body surface area (BSA), based on predictive nonlinear models. Thereby it becomes possible to estimate the expected liver volume and liver blood flow for the given anthorpomorphic information and with this to calculate the expected clearance for the person. We employed this method to predict population variability in GEC. Of special interest were the changes in aging.
‘The mechanistic parameters, namely liver volume and hepatic blood flow were described with

probability density functions (PDF) estimated from individual subject data (LMS, GAMLSS) approach. Since each PDF depicts the frequency of occurence of all expected values for each parameter in the population, the effects of multiple sources of uncertainty and variability were accounted for in the estimated distribution of GEC in the population.

Predicted vs. experimental data points are shown in Figure 5AB.

Population Variability Given a cohort with anthropomorphic features for the the individuals our model allows the prediction of expected distribution of GEC values for the individuals in the cohort. If the cohort is representative for the population, the population variability of liver function can be estimated. We estimate the population variability in the US population based on the NHANES cohort {NHANES} (Figure 5BC). Not only GEC and GECkg are predictied correctly, but other pairwise correlations like the dependency of liver volume and blood flow from age, bodyweight, height and BSA (see Figures supplement). The presented methods allows thereby an estimation of the distribution of liver function based on the variability of hepatic perfusion and liver volume in the population.

GEC in aging A significant negative correlation as observed between age and both liver volume and apparent liver blood flow above 30 years. The reduction in liver volume, apparent liver blood flow and perfusion may at least partly account for the decline in the clearance of many drugs undergoing liver metabolism, which has been noted to occur with aging in man {Wynne1989, Schnegg1986}. Also during childhood major absolute and relative changes per body weight occur in liver volume and bloodflow. To test if this underlying changes in liver morphology and perfusion can explain the age-dependent changes

Application of the model revealed that variability of galactose clearance in aging is mainly explained by changes in liver structure, perfusion and morphology during lifetime. These alterations with age have important implications for drug dosing.

TODO: discuss the effects of ultrastructur changes, only effects under low concentration clearance.

GEC classification

The predicted distribution of liver function (GEC) for an individual can be employed for an improved evaluation of the result of a liver function test. I.e. if the further the measured GEC from the expected GEC, the higher the probability for liver disease. This approach was evaluated retrospectively in a large cohort study {Fabbri1996} and with data available from the literature (see table ?), which measured GEC in combination with anthropomorphic information.

A classifier based on our predictive GEC model outperformed classifiers for liver disease based on logistic regression with GEC predictor (Figure 5E), and was comparable to more complex classifiers using GEC as well as age, gender, bodyweight and sex into account. The GEC prediction in combination with the classifier for liver disease was implemented in a web application allowing the presented calculation of GEC range and classification of liver disease based on the presented model (Figure 6). We demonstrate the application of a personalized multiscale model of the human liver providing improved evaluation of an established liver function

test.

- Earlier detection of liver disease, earlier intervention

GEC as predictor in survival of cirrhosis {Merkel1991}

This has important consequences for the evaluation of the functional capacity/reserve and the detection of impairments and disease, both crucial for organ evaluation in transplantation or in the calculation of proper drug dosage depending on age.

DISCUSSION

We have developed a multiscale. model that accounts for …

- explains a variety of emergent behaviors in terms of molecular interactions.
- *Our model accurately recapitulates a broad set of experimental data*
- *provides insights into several biological processes for which experimental assessment is not readily feasible, and enables ...*

mathematical modelling to make testable predictions and gain
insight about a biological system's behaviour.

The model includes the common key processes that lead to liver diseases, metabolism, perfusion and ultrastructure of the liver. The multiscale model's explicitly modeled tires of resolution provide information beyond that which can be obtained by independently exploring single scales in isolation.

A clear and immediate need exists for evidence-based guidance for the identification of people being at risk of liver disease, and follow-up in deteriotation / improvement of liver function.

Model assumptions

A mathematical model is always only a selective representation of reality. Certain model assumption had to be made due to lack of data and the boundaries of the model

- We could not retrieve correlation data between sinusoidal blood flows and ultrastructural parameters of the liver. The distributions of sinusoidal parameters were assumed statistically independent from each other.
- No changes in gene expression, protein levels were taken into account. Galactose metabolism and GEC are quit constant. Adult rats fed a 40% galactose diet for 5 days did not show an increase in GEC although 20 days on the diet resulted in a 20% increase in V_{max} suggesting that adaptive mechanisms are slow [Schirmer1986 ->18]. This lack of inducability and relatively constant V_{max} is desirable in clearance methodology as a fluctuating V_{max}/FK_m would certainly complicate clearance interpretations. An important part of the individual GEC is the actual protein expression of the key enzymes. With the availablity of omics data these can readily be integrated in the model to further improve personalized predictions of elimination rates.

  Results from rats fed low protein diets indicate that GEC in rats deprieved of dietary

protein is determined by the amount of hepatic protein. GEC was signifcantly decreased to appoximately half of control values with hepatic protein content reduced to the same extent {Vilstrup1976}

- Dispersion of dilution peaks in the large vessels and runtime differences were not modelled. It is assumed that no displacement occurs between reference intravascular and diffusible tracers in the large vessels: all displacement occurs in the exchanging vessels (sinusoids). The interrelations between whole-organ outflow reference and diffusible tracer curves will depend not only on the phenomena occurring within each sinusoid but also on the way the transit times in larger vessels and sinusoids are interrelated. Various combinations are possible, depending on the structure of the network and the kind of flow coupling in the system. The pattern corresponding to the liver was found to lie at a simple extreme in this possible spectrum [Rose1976, Goresky1970]. The distribution of out-flow transit times was found to correspond to the distribution transit of sinusoidal times in large transit times; the distribution of vessels was so compact that a single value could be assumed [Rose1976, Goresky1970] supporting the model assumption.
- The other question is if heterogeneity in sinusoidal blood flow and transit times exist. Goresky et al.1 previously have considered two models representing the extreme cases, i.e., no heterogeneity, and maximum heterogeneity in capillary transit times. Multiple indicator-dilution data from the liver fit the latter model well [Rose1976].
- Heterogeneity in local blood flow in the liver was not taken into account.
- Part of the model relies on predictive models of liver volume and bloodflow which were trained with trainingssets based on multiple studies. The predictions reflect this subset of data used for model fitting. Care was taken only to use data for Caucasian/Western individuals with normal bodyweight range and without any liver disease. Nonetheless the regression models reflect the used trainingssets.

Most of these assumptions are necessary to a lack of experimental data or the focus of the current modeling question. We see this model as a first draft. The model and all source code is made freely available under xxx licence and is available from.

Comparison to other liver models

- Höhme (no detailed blood flow & heterogeneity, no metabolism, based on rat data, no space of Disse, fenestraetion, no evaluation against multiple indicator data & at same time total rates)
- Chaloubh & other simple sinusoid models without flow heterogeneity (missing parameter distributions, only briding the gap to the sinusoidal unit, not possible to simulate the different effects of heterogenous variations of parameters, no scaling to liver)
  These models do not reflect the reality of highly heterogeneous bloodflow and liver on sinusoidal scale.
- Distributed models, simple clearance models (Bass, Keiding, …) -> no detailed metabolism, can not answer the effects on cell level (good approximations for many cases)
- Ricken & other porous media approaches (human cast model!) (only on lobulus level, no

modeling of actual ultrastructure, different approach for different questions, material-properties, stiffness)

All models fail in accounting … & and none could demonstrate clinical relevance.

Towards a virtual liver & future applications

Bridging the scales from cellular processes over the coupling of single cells within the tissue-architecture towards whole-organ models is a crucial step in understand physiological function of organs in the normal state and in pathophysiologies. Only by modelling the different scales explicitly the emerging behaviour on a liver scale can be properly understood.

- the systems biology approach, i.e. the interaction between biological experiments and mathematical modelling, is to be transferred to application-oriented liver research as a next step
- In order to use the understanding of these processes to develop novel treatment and prevention approaches, disease-relevant and, if possible, personalized multiscale models are to be derived.

Online Methods

The presented liver model is a multi-scale model comprising cellular scale on the level of single hepatocytes (Figure 1A), tissue scale on level of the sinusoidal unit (Figure 1B), a region of interest of the liver (lobulus, Figure 1C) and the liver in individual subjects and variability in the population (Figure 1D).

Availability of data and models

The complete source code of modelling and analysis is open source and available under GPL from https://github.com/matthiaskoenig/multiscale-galactose. All literature data sets are made accessible on request. The mathematical model on cellular and tissue-scale are published as SBML under creative commons (CC BY-SA 4.0) in the Supplementary Information as well as on Biomodels.org and JWS Online. A HTML representation of the information is available in the Supplement.

Numerical integration

The hepatocyte and sinusoidal unit models are kinetic model based on ordinary differential equation (ODE) systems. All numerical integrations were performed directly with the SBML with libRoadRunner v1.3 {Somogyi2014, Somogyi2015} with absolute and relative tolerances of 1E-6 on a computer cluster. libRoadRunner was extended to efficiently handle the large models via ...

All results 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. All literature based kinetic parameters were included in SABIO-RK {Wittig2012} (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).

Tissue scale - 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}.

Liver region of interest

The heterogeneity of sinusoidal units within a lobulus was modeled via Monte Carlo simulation of varying sinusoidal units based on experimental distributions for parameters of 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 v_{blood}). The output of a region of interest was calculated via integration of the results from N=1000 sinusoidal units. All 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 via $stdlog = \sqrt{log(1+\frac{std^2}{m^2})}$ and $mlog = log(\frac{m^2}{m^2+std^2})$. All parameters and references are given in Supplementary Table 4.

For the variation of perfusion, i.e. blood flow per tissue volume, the distribution of blood flows was scaled via $p_{scaled}(v_{blood})= p(f_{flow}*v_{blood})$ mit $f_{flow}=1$ corresponding to the experimental microcirculation.

Liver

To scale the output from region of interests to main things have to be taken into account. 1) The region of interests describe the parenchymal liver tissue. To account for whole liver function the non parenchymal volume of the liver, mainly consisting of large vessel volume, has to be taken into account. The parenchymal fraction of the liver is arround 80% ($f_{tissue}=0.8$).

The relatiionship between tissue perfusion $P_{tissue} = \frac{Q_{tissue}}{V_{tissue}} = \frac{\sum_{i=1}^{N} Q_{sinuit}}{\sum_{i=1}^{N} V_{sinunit}}$ and liver perfusion $P_{liver} = \frac{Q_{liver}}{V_{liver}}$ is given via $P_{liver} = \frac{Q_{tissue}}{V_{tissue}+V_{notissue}} = (\frac{1}{2-f_{tissue}})\frac{Q_{tissue}}{V_{tissue}} = (\frac{1}{2-f_{tissue}})P_{tissue}$

Via the relationship for normal perfusion of 1.2ml/min/ml an necessary adaption of the microcirculation of $f_{flow}=0.3$ results corresponding to a mean sinusoidal flow velocity of 81µm/s. This is still in the range of the experimentally obtained values. OPS values and microcirculation is taken on the surface of the liver, with partly larger arterial components and properly not representative of the whole liver.

Multiple indicator dilution curves

An indicator substances introduced into the blood flowing into the liver become dispersed in the effluent blood and the concentrations oft he substances in the effluent blood form an indicator dilution curve {Goresky1973}. The rapid injection of labeled red blood cells (a vascular indicator), labeled sucrose and albumin (extracellular references), and labeled galactose under various galactose concentrations into the portal vein in combination with rapidly sampled venous blood were simulated {Goresky1973, Goresky1983} were simulated. For comparison with the experimental data the catheter and nonexchangeable vessel transit time t_0 was estimated from the time of first appearance of radioactivity above background levels in the experimental and simulated dilution curves. Integration of the single a region of interest for the periportal output was done via the volume flow weighted average of the individual sinusoidal units

$$c^{s}_{venuos}(t) = \sum_{i=1}^{N} w_i c^{s}_{pp}(t) = \sum_{i=1}^{N} \frac{Q_{sinunit,i}}{\sum_{i=1}^{N} Q_{sinunit,i}} c^{s}_{pp}(t)$$

The model is a distributed model of flow based on parallel, non-interacting sinusoids joined at the venous terminus. The dispersion characteristics is due to the *a priori* incorporated experimental velocity and path length variations within the ensemble of sinusoids {Weiss1995}

Galactose Elimination

The galactose elimination (GE), the removal rate (R), the extraction ratio (ER) and the clearance (CL) were calculated from the blood flow Q_{tot} and the periportal and perivenous concentrations of galactose c^{gal}_{pp} and c^{gal}_{pv} using the following equations {Schirmer1986}

$$GE = Q\,(c_{pp}^{gal} - c_{pv}^{gal})$$
$$ER = \frac{(c_{pp}^{gal} - c_{pv}^{gal})}{c_{pp}^{gal}}$$
$$CL = Q\,\frac{(c_{pp}^{gal} - c_{pp}^{gal})}{c_{pp}^{gal}}$$

For the region of interest the sinusoidal units are integrated resulting in

$$GE = (c_{pp}^{gal} - c_{pp}^{gal})$$
$$R = Q\,(c_{pp}^{gal} - c_{pp}^{gal})$$
$$ER = \frac{(c_{pp}^{gal} - c_{pp}^{gal})}{c_{pp}^{gal}}$$
$$CL = Q\,\frac{(c_{pp}^{gal} - c_{pp}^{gal})}{c_{pp}^{gal}}$$

Liver in aging

The age dependent change in total liver volume, blood flow and perfusion are taken into account in the age-dependent GAMLSS curves. The additional changes in ultrastructure of the liver (pseudocapillarization) were modeled using the age-dependent change in fenestration, i.e. change in fenestration number per area (N_{fen}), and endothelial thickness (y_{end}) based on experimental data as input for the model predictions (supplementary information).
Practically, for different combinations of (N_{fen}, y_{end}) corresponding to certain ages the GEC per tissue and perfusion were calculated.

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 females $p_{sex=F,volLiver\sim age}$. Importantly, the observed population variability is part of the model.
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

$$p_k(volLiver) = p(volLiver|sex = S,\ age = A,\ bodyweight = B,\ height = H,\ bsa = BS) =$$
$$p_{sex=S,volLiver\sim age}(volLiver|age = A) \cdot$$
$$p_{sex=S,volLiverkg\sim age}(volLiver|age = A,\ bodyweight = B) \cdot$$

$$p_{sex=S,volLiver\sim bodyweight}(volLiver|bodyweight = B) \cdot$$
$$p_{sex=S,volLiverkg\sim bodyweight}(volLiver|bodyweight = B) \cdot$$
$$p_{sex=S,volLiver\sim height}(volLiver|height = H) \cdot$$
$$p_{sex=S,volLiverkg\sim height}(volLiver|height = H,\ bodyweight = B) \cdot$$
$$p_{sex=S,volLiver\sim bsa}(volLiver|bsa = BS) \cdot$$
$$p_{sex=S,volLiverkg\sim bsa}(volLiver|bsa = BS,\ bodyweight = B)$$

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

$$p_k(flowLiver|volLiver = V) = p(flowLiver|sex = S,\ age = A,\ bodyweight = B,\ bsa = BS) \cdot p_{sex=S,flowLiver\sim volLiver}(flowLiver|volLiver = V)$$
$$p_{sex=S,flowLiver\sim age}(flowLiver|age = A) \cdot$$
$$p_{sex=S,flowLiverkg\sim age}(flowLiver|age = A,\ bodyweight = B) \cdot$$
$$p_{sex=S,flowLiver\sim bodyweight}(flowLiver|bodyweight = B) \cdot$$
$$p_{sex=S,flowLiverkg\sim bodyweight}(flowLiver|bodyweight = B) \cdot$$
$$p_{sex=S,flowLiver\sim bsa}(flowLiver|bsa = BS) \cdot$$
$$p_{sex=S,flowLiverkg\sim bsa}(flowLiver|bsa = BS,\ bodyweight = B) \cdot$$
$$p_{sex=S,flowLiver\sim volLiver}(flowLiver|volLiver = V)$$

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 $p_k(volLiver)$ and $p_k(flowLiver|volLiver)$ the distribution of liver volumes, blood flows and metabolic function in people with the given anthropomorphic features can be 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 ≤ BMI ≤ 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).

Body surface area

Body surface was (BSA) is calculated via DuBois formula from bodyweight and height $BSA = 0.007184 \cdot bw^{0.725} \cdot h^{0.425}$ {Moesteller1987} .

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The model source code, training data, and results are freely available at https://github.com/matthiaskoenig/multiscale-galactose).

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