# A Computational Multi-Scale Model Enables Personalized Predictions of Altered Metabolic Functions in Distinct Regions of the Liver

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## ABSTRACT

The metabolic capacity of the liver is determined by both tissue architecture, blood perfusion and cellular (hepatocyte) metabolic performance which all may be altered in liver diseases. Taking the galactose elimination capacity (GEC) as an important non-invasive liver function parameter we developed a multi-scale model to decipher how the GEC is brought about as integral outcome of a huge number of liver lobuli differing in tissue and vessel geometry and sinusoidal blood flow rates. Using clinical data from a retrospective analysis of a large cohort study we demonstrate that our model outperforms conventional regression models in the prediction of individual variations of the GEC depending on age, gender and anthropomorphic measures. Using as model input quantitative hepatic CT perfusion measurements of normal and a fibrotic/cirrhotic human livers the model generates 3D-functiograms highlighting the heterogeneity with which different regions of the liver contribute to the overall GEC. We conclude that extension of the proposed multi-scale model to other non-invasive liver function tests may provide a comprehensive spatially resolved *in silico* image of liver functionality that improves the early diagnosis of an ongoing liver disease and supports decision making in liver surgery.

## INTRODUCTION

The liver is the central metabolic organ of our body performing hundreds of metabolic functions including the homeostasis of numerous plasma metabolites, production of the bile, detoxification of xenobiotics, medical drugs and endogenous waste products. Despite progress in the diagnosis and management of liver diseases, yet approximately 29 million people in the European Union suffer from a chronic liver condition with underlying mechanism often being unclear {Blachier2013}. Liver function is the result of complex interplay of hepatic morphology, blood perfusion and metabolism across multiple spatial domains spanning from the single-cell level up to the entire organ {Rappaport1979}. Computational models are uniquely positioned for the analysis of such complex systems owing to their capacity to integrate knowledge on molecular processes and structures available for different spatial and temporal scales [1]

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### Liver Architecture

Liver architecture is uniquely homogenous in that it can be virtually generated by about one million instantiations of a basic structural unit, the liver lobuli, 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 sinusoid surrounded by a layer of hepatocytes separated by the space of Disse, forms the smallest functional unit of the liver (Figure 1). 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 {Cogger2003}.

### Age-related Changes of Tissue Architecture and Hemodynamics

The percentage of deaths attributed to liver disease increases dramatically in humans beyond the age of 45 years {Schmucker2005}. Physiologic changes in the elderly include: decline in liver volume, liver blood flow and liver function {Anantharaju2002, Wynne1989}. In addition, characteristic changes in ultrastructure termed pseudocapillarization occur, 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}. It is not clear to which extent alterations in vessel architecture, microvascular haemodynamics and diffusive nutrient and oxygen supply are contributing factors to the age-related decline of liver functions {Vollmar2002}.

### 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 non-invasive liver 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}, is an established test of liver function applied 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}.

### Multiscale-Model of Hepatic Galactose Metabolism - Objectives

Systems-level computational approaches are required to elucidate the complex interaction of organ structure, perfusion, and metabolism and to understand how these different levels influence liver function, here the clearance of galactose and GEC. Mathematical models are uniquely positioned to capture the connectivity between different temporal and spatial 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 impact of age-dependent alterations in organ perfusion, liver volume and tissue ultrastructure on GEC and thus to better define the expected normal range of the GEC given the gender, age and anthropomorphic measure of the individual patient, iv) can predict intra-organ heterogeneity of metabolic functions resulting from regional variations of tissue parameters and perfusion rates and thus v) can highlight already present regional metabolic deficits at still normal overall metabolic function of the liver.

### Model Structure and Modeling Strategy

The multi-scale model of the human liver combines a detailed kinetic model of cellular galactose metabolism (Figure 1A) with a tissue-scale perfusion model of the sinusoid (Figure 1B). All model equations and the decription of all model variables is given in supplement ???

**Cell - Galactose Metabolism** - In hepatocytes, galactose is converted to UDP-glucose (udpglc) in three 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}. The major fraction of galactose enters glycolysis as glucose-1 phosphate (glc1p), as small fraction of udpgal is used for the galactosylation of glycoproteins and glycolipids {Novelli2000}. Temporal changes of the metabolites involved in the cellular galactose metabolism are modelled by ordinary differential equations. The enzymatic rate laws were choo

**Sinusoidal Unit (SU)** – A single liver lobule is composed of about 1000 so-called sinusoidal units (SUs). A SU is constituted by a single sinusoid which is aligned by a monolayer of 20 – 25 hepatocytes that are separated from the sinusoid by the space of Disse. In our model the SU is modelled as a grid of compartments each one representing a distinct spatial tissue and cell volume. Transport of cells and metabolites within the sinusoidal blood stream is assumed to be convective, exchange of metabolites and oxygen between the blood stream and the hepatocytes is treated as diffusional transport. The statistical distribution of tissue parameters (blood flow rate, area of sinusoid fenestrae, thickness and length of sinusoids, size of hepatocytes, extension of the space of Disse) was based on electron-micrographic data (Ref.).

**Lobule Scale** – In our module we define the lobule as an ensemble of 1000 SUs (see Fig ?). The metabolic capacity and blood perfusion of the lobule is calculated by integrating the contribution of the constituting 1000 SUs each one defined by a set of tissue parameters and a blood flow rate sampled from statistical distribution functions. These computations are performed at different concentrations of galactose in the sinusoidal blood and different distributions of sinusoidal blood flow rates to account for heterogeneous perfusion of different regions of the liver parenchyma.

**Organ Scale** - The metabolic function of the whole liver is modelled by averaging across an ensemble of ??? (how many?) lobules and subsequent extrapolation of these results to the organ scale by taking into account the liver volume of the individual. If no data on regional blood perfusion rates are available (e.g. through CT perfusion measurements) the blood flow rate of the lobules considered in the averaging procedure is rescaled by a constant factor to yield the total organ perfusion rate of the individual

**Personalized Liver Function** – If available, the size (volume) and the perfusion rate of the patient’s liver are used for the computations on the organ scale. If these items are not known the computations on organ scale are repeatedly carried by choosing liver size and perfusion rate from statistical distributions constructed on the basis of clinical data on organ perfusion rates and liver volume determined in individuals of different age and body mass (see supplement ???).

### **RESULTS**

### Multiple Dilution-Indicator Curves

In a first step the model was validated against 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 macromolecules and blood cells are normally excluded from the space of Disse. Low-molecular weight compounds as galactose or sucrose can be exchanged between sinusoidal blood and hepatocytes by diffusional transport. The outflow concentration of each tracer compound is divided by the total injected one, providing a normalized value, the outflow fraction per volume (ml). Integration across the output of a statistically representative manifold of sinusoidal units yields the total output of the liver that has to be compared with measured dilution curves.

At low blood galactose concentrations, the labeled galactose appears at the outflow with labeled sucrose, 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 in the venous outflow. Of note, none of the experimental data was used for model fitting, i.e. all model parameters result from geometric constraints inferred from ultra-structural tissue data and the physiochemical properties of the substances transported within the sinusoid and the space of Disse. Only the galactose exchange rate between hepatocytes and the space of Disse was adjusted to yield the observed total galactose elimination per volume tissue.

### Assessment of Functional Heterogeneity Within and Between Sinusoids of the Normal Liver

Our multiscale-model allows to compute the temporal profile of galactose in different parts of the sinusoidal unit (Fig. 3). These computations reveal large gradients along the periportal – perivenous axis of a single sinusoid. By explicitly accounting for heterogeneity in sinusoidal blood flow and tissue parameters (Figure 2A) we also analyzed the variability of temporal galactose profiles within a lobule (Figure 2D). Our calculations show that local concentrations of galactose within a lobule may differ up to a factor of ???. In contrast, the average galactose time profile of the lobule shows only little variability (Fig. ???). Taken together, our simulations suggest a surprisingly large variability of galactose concentrations in the spatial range of a few millimeters

### Hepatic Galactose Elimination

Hepatic galactose clearance (GC) and the extraction ratio (ER) of a normal human liver were calculated as described in Methods for various organ perfusion rates and various periportal galactose concentrations. As shown in Fig. 4AB, the model correctly reproduces the observed saturation of GC and the dependency of the ER of galactose of the organ perfusion rate. The GE follows a hyperbolic saturation kinetics {Keiding1973, Keiding1976} with a concentration-dependent (first-order) elimination phase at low galactose concentrations and a clearance maximum, the so-called galactose elimination capacity (GEC), at high periportal galactose concentrations (zero-order phase) {Schirmer1986}. Important determinants of galactose clearance are the metabolic capacity of the hepatocyte and the liver blood flow {Winkler1978}.

At *low plasma galactose levels* the clearance is determined by the hepatic blood flow, Therefore, galactose clearance at low galactose load 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士3 {Keiding1988}.

At *high plasma galactose levels* the clearance becomes limited by the metabolic capacity of the hepatocyte (Figure 4C). Indeed, hepatic vein catheterization at high plasma galactose concentrations revealed a constant hepatic arterial-hepatic venous concentration difference {Tygstrup1954, Tygstrup1958} indicating that hepatocytes participate maximally in the removal of galactose. Thus, at high galactose load, the test has been regarded as a measure of the functioning liver cell mass (Lm) {Tygstrup1966}.

### Personalized GEC prediction

Reference ranges of diagnostic parameters defining the range of values considered to be “normal” play an important role in medical practice {Cole2009}. Here we used the multi-scale model to compute reference ranges for the diagnostic parameter galactose elimination capacity (GEC) thereby taking into account liver size and the hepatic blood perfusion rate of the individual. As these two cardinal input values are available in rare cases only, we constructed phenomenological regression functions to estimate liver size and perfusion rate from a set of easily obtainable patient-specific parameters (PSPs): Age, gender, bodyweight, height and body surface area. From the statistical distribution of the residuals between observed values and the values predicted by the regression functions one may derive probability distributions (PDs) of liver size and perfusion rate at given values of PSPs.

The GEC of a given patient was recurrently computed (??? how often) while using the PSI to randomly choose liver volume and organ perfusion rate from a multivariate statistical distribution (probability density function – PDF) that was constructed as described in Materials. This procedure yields a statistical distribution of GEC values which can be used to define a patient-specific reference range of “normal” GEC values encompassing 95% of all computed GEC values. In cases where the PSI for a patient is incomplete, i.e. only a subset of parameters are available (e.g. only age and gender), …

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

**Prediction of Population Variability** We applied the procedure to predict personalized GEC values described above to two differenta cohort of individuals with known PSI anthropomorphic features for 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 GEC per body weight (GEC\_kg) are predicted 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 blood flow. 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 ultrastructure 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

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.

*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 Vmax suggesting that adaptive mechanisms are slow [Schirmer1986 ->18]. This lack of inducability and relatively constant Vmax is desirable in clearance methodology as a fluctuating Vmax/FKm 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.

#### 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 (Vmax) 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 Nc 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 (rsubstance≤rfen) 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 ra is given as actual diffusion Da relative to unhindered Diffusion Da,0 with radius of the substance ra

and pore radius rfen as {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 Lsin, sinusoidal radius ysin, width space of Disse ydis, hepatocyte sheet thickness ycell) and microcirculation (sinusoidal blood flow vblood). 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 ysin, vblood and ycell were fitted based on maximum-likelihood method for uni-variate distributions . For Lsin and ydis the log-normal parameters were calculated from reported mean m and standard deviation std via and . 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 pscaled(vblood)= p(fflow\*vblood) mit fflow=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% (ftissue=0.8).

The relatiionship between tissue perfusion and liver perfusion is given via

Via the relationship for normal perfusion of 1.2ml/min/ml an necessary adaption of the microcirculation of fflow=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 t0 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

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 Qtot and the periportal and perivenous concentrations of galactose andusing the following equations {Schirmer1986}

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

### 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 (Nfen), and endothelial thickness (yend) based on experimental data as input for the model predictions (supplementary information).

Practically, for different combinations of (Nfen, yend) 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.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 pk(volLiver) for the subject k with sex=S, age=A, bodyweight=B, height=H and bsa=BS

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

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 (flowLiverk and volLiverk)via the metabolic functions calculated for regions of the liver.

GECk = fGEC\_per\_volLiver(flowLiverk/volLiverk) \* volLiverk

Via Monte Carlo simulation, i.e. repeated sampling from the individualized probability distributions and 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 {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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