# A Multi-scale Model of Human Liver Metabolism: Individualized Prediction of Hepatic Function based on Galactose Elimination Capacity

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

1Department of Computational Systems Biochemistry, University Medicine Charité Berlin, D-10117 Berlin, Germany

2Department of Internal Medicine, Aging and Nephrological Diseases, University of Bologna, I-40138 Bologna, Italy

3Department of Hepatology and Gastroenterology, Aarhus University Hospital, DK-8000 Aarhus, Denmark

4Biocomplexity Institute, Indiana University, Simon Hall MSB1, Bloomingtom, IN 47405

**Running Title:** Hepatic galactose metabolism in aging

**Keywords:** Virtual Liver, aging, galactose metabolism, GEC

**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](mailto:matthias.koenig@charite.de)

## ABSTRACT

The liver is the central metabolic organ of the body, and malfunction is a major contributor to disease. Hepatic function is a result of the complex interplay of organ structure, perfusion, and metabolism, and thus systems-level computational approaches are required to elucidate and understand underlying principles.

We have constructed a physiology-based model of the Human liver bridging the scales from single-cell metabolism over tissue representation of ultra-structure and micro-circulation to the whole-organ integrated with perfusion. Herein we describe the model application to galactose metabolism and the personalized evaluation of liver function based on individual anthropomorphic information.

The modeling of population variability in liver function on NHANES cohort.

These results highlight some of the applications enabled by the model. The establishment of this Virtual Liver represents an important step toward multi-scale human systems biology and individualization of liver function tests.

## INTRODUCTION

### Liver architecture

The liver is the central in maintaining the homeostasis of numerous plasma metabolites, clearance of substances and detoxification of xenobiotics. The liver architecture is unique within the body in that hepatic functionality is parallelized across a multitude of structural similar hexagonal subunits, the lobuli. Within a single lobule a network of capillaries, the so-called liver sinusoids, connect periportal regions, supplied via portal vein and hepatic artery, with the perivenous region, drained by the central vein {Sasse1992}. Sinusoids, separated from the surrounding hepatocytes via the space of Disse, form the smallest functional unit of the liver (Figure 1). Liver function is the result of the complex interplay between morphological structure, perfusion and metabolism on single cell level.

### Hepatic Galactose Metabolism

The liver is the most important organ for the whole-body metabolism and clearance of galactose {Bernstein1960, Berry2000, Segal1971}. The galactose elimination capacity (GEC) is an established test of liver function reflecting the functional hepatic mass and liver volume {Marchesini1988, Schirmer1986, Tygstrup1966}. Impairment of the liver and hepatocytes by diseases like cirrhosis {Henderson1982, Jepsen2009} or intoxication {Vilstrup1983} is commonly associated with impaired galactose clearance.

Galactose clearance on organ level is a consequence of galactose metabolism of individual hepatocytes consisting of three main enzymatic steps (Figure 2), often referred to as Leloir pathway: 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}.

Surprisingly, 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 closes this gap by presenting a kinetic model of the galactose metabolism in human hepatocytes.

### Multiscale-Model

On top, in order to understand how the successive dilution or concentration of metabolites along the sinusoidal blood flow from the periportal to the perivenous site influences the cellular metabolism requires a tissue-scale model that combines spatial gradients of metabolite concentrations with the cellular metabolism of individual liver cells. Finally, for the correct interpretation of results obtained in the galactose tolerance test it is necessary to take into account regional variations of the blood flow and in the architecture of sinusoids. Thus, for fully understanding the metabolic input-output relationship of the liver it needs to bridge the spatial scales from single hepatocyte metabolism over tissue-scale functional units of sinusoids to the whole-organ level.

For the individualized evaluation of galactose clearance tests the model is personalized based on antropomorphic data, namely age, gender, bodyweight, height and body surface area (BSA).

### GEC as Liver function test

“Quantitation of liver function is necessary to assess the degree of liver impairment, to objectively evaluate response to treatment and to select transplant recipients (1). Most of the commonly used quantitative tests, such as galactose-elimination capacity (2), sulfobromophthalein clearance (3) or antipyrine clearance (4) measure the disappearance of a test compound from blood. From these measurements, clearance is calculated and functional capacities are inferred without knowledge of details of the hepatic metabolism.” {Dufour, 1992 #160}

The galactose elimination capacity (GEC) is defined as the rate with which a given plasma load of galactose is cleared. The GEC depends on the amount of metabolically active liver cell mass and the liver perfusion (Marchesini, et al., 1988; Tygstrup, 1966): The GEC is an accepted test of liver function and has been measured in numerous studies [Schirmer -> 5, 9, 17, 22, 41, 78, 79, 82, 83, 88, 96]. [Schirmer1986 -> 17,82, 83, 88, 96].

“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) (28). This concept has been supported by the proportional reductions in galactose elimination capacity and BSP elimination in patients with liver diseases (7,20)” {Ducry, 1979 #161}

Hepatic galactose elimination follows Michaelis-Menten saturation kinetics [43, 50, 70]; Its hallmarks are an early, concentration-dependent (first-order) elimination phase followed by a definable clearance maximum (GEC) at higher concentrations (zero-order phase).

### Liver in aging

The percentage of deaths attributed to liver disease increases dramatically in humans beyond the age of 45 years {Schmucker2005}. Aging is characterized by normal progressive decline in functions that, cumulatively, diminish a cell’s, organ’s or organism’s capacity to respond to intrinsic or extrinsic stimuli. Physiologic changes known to play a role in the pharmacokinetics of a drug in the elderly include: decline in total body mass, liver volume, liver blood flow and liver function {Anantharaju2002, Wynne1989}. Also the ultrastructure of the liver changes with aging:

Pseudocapillarization, characterized by defenestration, thickening of the endothelium, and deposition of basal lamina and extracellular matrix in the space Disse, is a widespread change in aging {McLean2003, LeCouteur2001 , Cooger2003}. 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 in the liver sinusoids and space of Disse 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 differences in microangio-architecture, cellularity, microvascular haemodynamics and nutritive tissue perfusion of the liver are contributing factors of age-related susceptibility of the liver {Vollmar2002}.

### Relevance

Understanding the interplay of structure, perfusion, metabolism to create function on multiple scales. Especially, what effects have alterations in structure and perfusion in aging on metabolic function tests. What are the effects of individual differences in liver volume and blood flow.

This has important consequences for better evaluation of the functional capacity/reserve of the liver, in the detection of abnormalities based on individualized reference ranges, for the evaluation of liver function for liver transplantation or to calculate proper drug dosage depending on age.

## RESULTS

### Multiscale liver model

We present a multiscale mathematical model bridging the scales from individual cellular processes to the level of the entire organ. The model can be used to describe physiology, morphology and function of the human liver by integrating hepatic metabolism with perfusion and liver structure. The model combines detailed kinetic models of cellular metabolism with a tissue-scale perfusion model of the sinusoid. 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). 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. 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.

The model integrates and predicts experimental data on multiple temporal and spatial scales: i) multiple indicator dilution curves; ii) extraction and clearance of galactose depending on perfusion and galactose load; iii) individual GEC, population variability in GEC and the dependency of GEC on aging; iv) classification of liver disease based on predicted GEC. Personalized multiscale models are generated based on individual antropomorhic information in combination with fitted relationships describing the dependencies of hepatic volume and blood flow on anthropomorhic features. We demonstrate possible clinical application of this systems biology approach by outperforming classical approaches of classification of liver disease in a retrospective analysis of a large cohort study.

### Multiple Dilution-Indicator Curves

In a first step the model was validated multiple-indicator dilution curves. None of the data was used for model fitting, all model parameters result from the geometric constraints of the system and the physio-chemical properties of the substances transported within the sinusoid and the space of Disse. The model performance was validated based on published multiple-indicator dilution curves in human [REF] and dog {Goresky1973}.

Multiple indicator dilution curves for a multitude of substances could be replicated (Figure 3). Very interestingly 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, resulting in a delayed appearance of the substances perivenious.

The single-injection, multiple-indicator dilution approach provides a method to determine the composition of the liver and the rates of hepatic processes (Goresky, et al., 1973). Labeled red blood cells (RBC) are used as vascular reference. Larger materials are excluded from the space of Disse. The model of Goresky provides a realistic alternative to the too simple lumped compartmental descriptions of the liver classically utilized in pharmacokinetics. It provides a framework such that each curve can be directly compared with each other, the outflow concentration of each tracer is divided by the total injected, providing a normalized value, an outflow fraction per ml.

At low blood galactose concentrations, the labeld galactose appears at the outflow with labeld 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. ([Goresky, et al., 1973)](https://docs.google.com/document/d/1Yvd5FP4MM-NfLK45Rs0v4TScY112jSsxS0c-MCFUnp4/edit#heading=h.1fob9te).

* extreme heterogeneity on the lobulus & sinusoidal units (network for clearance, broad spectrum of parameters
* 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. In addition the altered galaactose tracer peaks under varying galactose background provides testing of the model

### Galactose Elimination Capacity

Via integration of the heterogenous sinusoidal units the hepatic galactose clearance , extraction and the galactose elimination capacity for given perfusion per volume liver is calculated. The model reproduces reproduces the observed saturation in galactose clearance, the dependency of the extraction ration of galactose on the perfusion.

The model of hepatic galactose metabolism integrated within the sinusoidal units was validated on the basis of measured GEC curves under normal conditions. The model reproduced the observed saturation kinetics for galactose elimination (low/high). The dependence of the hepatic galactose elimination rate follows a Michaelis-Menten saturation kinetics [Schirmer1986 -> 43, 50, 70]; with a quasi-linear concentration-dependent (first-order) elimination phase followed by a clearance maximum (GEC) at higher concentrations (zero-order phase).

**Low galactose (EHBF)** Galactose Clearance at low concentrations (GCLC) 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 [35]. The most extensive work on galactose elimination kinetics was done by Keiding and co-workers [43-45, 48, 50, 85, 99]. The “sinusoidal perfusion model” they developed provides a sound theoretical justification for using galactose clearance at low concentrations to estimate EHBF [44, 45, 48, 50, 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 (a) it is kineticially simple 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.

**High galactose (GEC)** 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 (See Figure GEC curves ~ perfusion for high and low galactose concentration). Hepatic vein catheterization at high concentrations revealed a constant hepatic arterial-hepatic venous concentration difference [Schirmer1986 -> 87,88].

### Individual GEC prediction

**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.

**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 {} (Figure 5). 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 therby an estimation of the distribution of liver function based on the variability of hepatic perfusion and liver volume in the population.

### 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 retrospectivly in a large cohort study and with data available from the literature (see table ?), which measured GEC in combination with anthropomorhic information. A classifier based on our predictive GEC model outperformed classical logistic regression between GEC and liver disease, even when age and bodyweight where included in the logistic regression.

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.

*Improved liver function tests*

We demonstrate the application of a personalized multiscale model of the human liver providing improved evaluation of an established liver function test.

## Discussion

“The removal of substances from blood by hepatic clearance is influenced by three factors: the intrinsic elminimation capacity (hepatocyte function), hepatic extraction and liver blood flow. Galactose clearance in the blood concentration range of 0 to 0,55mmol/l measures clearance at infusion rates one fith to one thenth of intrinsic elimination capacity, is virtually independent of hepatic extraction and is thus a flow-dependent clearance.”{Henderson, 1983 #86}

Quantitative assessment of liver function.

Flow dependent clearance.

The model includes the common key processes that lead to liver diseases, metabolism, perfusion and ultrastructure of the liver.

### Metabolic changes & Genexpression, Individual levels of metabolism

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.

*Model assumptions*

A mathematical model is always only a selective representation of reality. The results have to be evaluated in the context of the underlying assumptions of the model. Many of these assumptions are due to a lack of experimental data.

* For instance, the distributions of sinusoidal parameters were assumed independent from each other. As soon as correlation data becomes available the model an be upated.

#### Dispersion of dilution peaks in the large vessels and runtime differences were not modelled. Relationship vascular tree and sinusoid transit times ? 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. Thus it was possible to derive a test for the single-sinusoid modeling. If, after a common transit time in large vessels, the sinusoidal transit time for each diffusible label in the liver is increased by the ratio of its total-to-accessible sinusoidal vascular space, then it should be possible to reverse this flow-limited delay effect in the curve for each diffusible label.

#### 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 very well [Rose1976].

* Heterogeneity in local blood flow in the liver was not taken into account.

### Comparison to current multiscale models of liver

Höhme (no detailed blood flow, no metabolism)

Chaloubh (missing parameter distributions, only briding the gap to the sinusoidal unit, not possible to simulate the different effects of heterogenous variations of parameters.

Galactose-Clearance Modelle – no detailed description of metabolism, no bridging to whole liver function

Ricken, porous media

### Selection bias

The prediction of individual liver volumes & flows is based on selected available studies for the correlation. 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.

A mayor problem is the availability of individual subject data and the willigness to share this data. So are for liver volumes a multitude of studies available (REFS), but only Heinemann1999 was able to retrieve the data and willing to share the information. This individual subject data is indespensible for the creation of individualized models. The availble data relating liver volumes with liver blood flow in aging is limited on a single study. Incentives for data sharing are more than overdue.

### Embedding in whole body model

For the galactose metabolism it is sufficient to model the liver, due to its main role in galactose clearance. The model only describes the one-time pass through the liver without recirculation of uncleared galactose. Herefore, it would be necessary to model the systemic circulation.

Multiple galactose peaks

Reference ranges & improved function tests

For many purposes, knowledge of the average value or the range of plausible values for model parameters is sufficient; however, for estimating interindividual differences in clearance, knowledge of the distributions of input parameters in the population is essential.

‘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 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.

### 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.

We showed the applicability of the approach in a clinical scenario by using the clinical measured liver function via GEC as an ouptut.

## 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 with libRoadRunner {Somogyi2014} with absolute and relative tolerances of 1E-6 on a computer cluster. libRoadRunner was extended to efficiently handle the very 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} .

## ACKNOWLEDGEMENT

This work was supported by the Federal Ministry of Education and Research (BMBF, Germany) within the Virtual Liver Network (VLN grant number 0315741). Special thanks for access to individual subject data to H. Wynne {Wynne1989}, A. Heinemann {Heinemann1999}, G. Cattermole {Cattermole2010}. Thanks to A. Somogyi {Somogyi2014} for the technical support with RoadRunner, SABIO-RK for integration of the kinetic parameters in their database, in particular M. Golebiewski, R. Kenia and U. Wittig, to T. Czauderna for help with SBGN and SBGN-ED {Czauderna2010} and the SBML community for their continuous support and help.

The authors declare no commercial or financial conflict of interest.

## REFERENCES

Anantharaju, Abhinandana, Axel Feller, and Antonio Chedid. "Aging liver." *Gerontology* 48.6 (2002): 343-353.

Bernstein, L.M., et al. (1960) The blood galactose disappearance curve as a test of liver function, Gastroenterology, 39, 293-304.

Berry, G.T., et al. (2000) Galactose breath testing distinguishes variant and severe galactose-1-phosphate uridyltransferase genotypes, Pediatric research, 48, 323-328.

Bosch, A.M., et al. (2002) Clinical features of galactokinase deficiency: a review of the literature, Journal of inherited metabolic disease, 25, 629-634.

Cattermole, Giles N et al. "The normal ranges of cardiovascular parameters in children measured using the Ultrasonic Cardiac Output Monitor." *Critical care medicine* 38.9 (2010): 1875-1881.

Centers for Disease Control and Prevention (CDC). National Center for Health Statistics (NCHS). National Health and Nutrition Examination Survey Data (NHANES). Hyattsville, MD: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, 1999-2012, http://www.cdc.gov/nchs/nhanes/nhanes\_questionnaires.htm.

Cogger, Victoria C et al. "Hepatic sinusoidal pseudocapillarization with aging in the non-human primate." *Experimental gerontology* 38.10 (2003): 1101-1107.

Cole, Timothy J, and Pamela J Green. "Smoothing reference centile curves: the LMS method and penalized likelihood." *Statistics in medicine* 11.10 (1992): 1305-1319.

Czauderna, T., Klukas, C. and Schreiber, F. (2010) Editing, validating and translating of SBGN maps, Bioinformatics, 26, 2340-2341.

Fridovich-Keil, J.L. (2006) Galactosemia: the good, the bad, and the unknown, Journal of cellular physiology, 209, 701-705.

Goresky, C.A., Bach, G.G. and Nadeau, B.E. (1973) On the uptake of materials by the intact liver. The transport and net removal of galactose, The Journal of clinical investigation, 52, 991-1009.

Goresky, C. A. (1983). Kinetic interpretation of hepatic multiple-indicator dilution studies. *Am. J. Physiol*, *245*(1), G1-12.

Heinemann, Axel et al. "Standard liver volume in the Caucasian population." *Liver transplantation and surgery* 5.5 (1999): 366-368.

Henderson, J.M., Kutner, M.H. and Bain, R.P. (1982) First-order clearance of plasma galactose: the effect of liver disease, Gastroenterology, 83, 1090-1096.

Jepsen, P., et al. (2009) The galactose elimination capacity and mortality in 781 Danish patients with newly-diagnosed liver cirrhosis: a cohort study, BMC gastroenterology, 9, 50.

Keppler, D., Rudigier, J. and Decker, K. (1970) Trapping of uridine phosphates by D-galactose in ethanol-treated liver, FEBS letters, 11, 193-196.

Knop, J.K. and Hansen, R.G. (1970) Uridine diphosphate glucose pyrophosphorylase. IV. Crystallization and properties of the enzyme from human liver, The Journal of biological chemistry, 245, 2499-2504.

Konig, M., Holzhutter, H.G. and Berndt, N. (2013) Metabolic gradients as key regulators in zonation of tumor energy metabolism: A tissue-scale model-based study, Biotechnology journal, 8, 1058-1069.

Le Couteur, D. G., Fraser, R., Cogger, V. C., & McLean, A. J. (2002). Hepatic pseudocapillarisation and atherosclerosis in ageing. *The Lancet*, *359*(9317), 1612-1615.

Le Novere, N., et al. (2009) The Systems Biology Graphical Notation, Nature biotechnology, 27, 735-741.

Leslie, N.D. (2003) Insights into the pathogenesis of galactosemia, Annual review of nutrition, 23, 59-80.

Marchesini, G., et al. (1988) Galactose elimination capacity and liver volume in aging man, Hepatology, 8, 1079-1083.

McLean, Allan J et al. "Age‐related pseudocapillarization of the human liver." *The Journal of pathology* 200.1 (2003): 112-117.

Mosteller, RD. "Simplified calculation of body-surface area." *The New England journal of medicine* 317.17 (1987): 1098.

Novelli, G. and Reichardt, J.K. (2000) Molecular basis of disorders of human galactose metabolism: past, present, and future, Molecular genetics and metabolism, 71, 62-65.

Pappenheimer, J. R. (1953). Passage of molecules through capillary walls. *Physiol. Rev*, *33*(3), 387-423.

Petry, K.G. and Reichardt, J.K. (1998) The fundamental importance of human galactose metabolism: lessons from genetics and biochemistry, Trends in genetics : TIG, 14, 98-102.

Renkin, E. M. (1954). Filtration, diffusion, and molecular sieving through porous cellulose membranes. *The Journal of general physiology*, *38*(2), 225-243.

Sasse, D., Spornitz, U.M. and Maly, I.P. (1992) Liver architecture, Enzyme, 46, 8-32.

Schadewaldt, P., et al. (2000) Analysis of concentration and (13)C enrichment of D-galactose in human plasma, Clinical chemistry, 46, 612-619.

Schirmer, W.J., et al. (1986) Galactose clearance as an estimate of effective hepatic blood flow: validation and limitations, The Journal of surgical research, 41, 543-556.

Schmucker, Douglas L. "Age-related changes in liver structure and function: implications for disease?." *Experimental gerontology* 40.8 (2005): 650-659.

Schnegg, Marianne, and Bernhard H Lauterburg. "Quantitative liver function in the elderly assessed by galactose elimination capacity, aminopyrine demethylation and caffeine clearance." *Journal of hepatology* 3.2 (1986): 164-171.

Segal, S. and Rogers, S. (1971) Nucleotide inhibition of mammalian liver galactose-I-phosphate uridylyltransferase, Biochimica et biophysica acta, 250, 351-360.

Somogyi, Andi, Simulation of electrochemical and stochastic systems using just in time compiled declarative languages, doctoral thesis (2014)

Stasinopoulos, D Mikis, and Robert A Rigby. "Generalized additive models for location scale and shape (GAMLSS) in R." *Journal of Statistical Software* 23.7 (2007): 1-46.

Tang, M., et al. (2012) Correlation assessment among clinical phenotypes, expression analysis and molecular modeling of 14 novel variations in the human galactose-1-phosphate uridylyltransferase gene, Human mutation, 33, 1107-1115.

Timson, D.J. (2005) Functional analysis of disease-causing mutations in human UDP-galactose 4-epimerase, The FEBS journal, 272, 6170-6177.

Timson, D.J. and Reece, R.J. (2003) Functional analysis of disease-causing mutations in human galactokinase, European journal of biochemistry / FEBS, 270, 1767-1774.

Timson, D.J. and Reece, R.J. (2003) Sugar recognition by human galactokinase, BMC biochemistry, 4, 16.

Tyfield, L. and Walter, J. (2002) Galactosemia. In Scriver, C., et al. (eds), The Metabolic and Molecular Bases of Inherited Disease. McGraw-Hill, New York.

Tygstrup, N. (1966) Determination of the hepatic elimination capacity (Lm) of galactose by single injection, Scandinavian journal of clinical and laboratory investigation. Supplementum, 18, 118-125.

Vollmar, Brigitte et al. "In vivo quantification of ageing changes in the rat liver from early juvenile to senescent life." *Liver* 22.4 (2002): 330-341.

Villeneuve, J.P., et al. (1996) The hepatic microcirculation in the isolated perfused human liver, Hepatology, 23, 24-31.

Vilstrup, H. (1983) Effects of acute carbon tetrachloride intoxication on kinetics of galactose elimination by perfused rat livers, Scandinavian journal of clinical and laboratory investigation, 43, 127-131.

Walter, J.H., et al. (1999) Generalised uridine diphosphate galactose-4-epimerase deficiency, Archives of disease in childhood, 80, 374-376.

Weiss, M. (1997). A note on the interpretation of tracer dispersion in the liver. *Journal of theoretical biology*, *184*(1), 1-6.

Wisse, E., et al. (1985) The liver sieve: considerations concerning the structure and function of endothelial fenestrae, the sinusoidal wall and the space of Disse, Hepatology, 5, 683-692.

Wittig, U., et al. (2012) SABIO-RK--database for biochemical reaction kinetics, Nucleic Acids Res, 40, D790-796.

Wynne, Hilary A et al. "The effect of age upon liver volume and apparent liver blood flow in healthy man." *Hepatology* 9.2 (1989): 297-301.