A Virtual Liver Model for Human Galactose Metabolism: Individualized Predictions of Liver Function

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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. 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.
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).

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

We have constructed a multi-scale model of the Human liver bridging the scales from single-cell metabolism over tissue representation to the whole-organ. The model is physiology and function based The model combines detailed kinetic models of cellular metabolism with a tissue-scale perfusion model of the sinusoid. The metabolic capacity of the whole liver for individual subjects is modelled by integrating the heterogeneous contributions of sinusoids differing in blood-flow rates and tissue-architecture via liver function.
The metabolic capacity of the whole liver is modelled by integrating the heterogeneous contributions of sinusoids differing in blood-flow rates and tissue-architecture via liver function.
Here, we present such a multi-scale model of the hepatic galactose metabolism. The grounding of this model is a detailed kinetic model of the cellular galactose metabolism. This 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). Finally, liver metabolism was modelled as weighted average across the contribution of tissue-scale models with differing blood flow, tissue geometry and cellular metabolic capacity.

- 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

Multiple Dilution-Indicator Curves

The model was validated on the basis of in vivo measured multiple indicator-dilution curves in dog and human und PET-data (human) of galactose metabolism.
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.

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].

Galactose Elimination Capacity (GEC)

“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}

“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}

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].

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]. The dependence of the hepatic galactose elimination rate follows a Michaelis-Menten saturation kinetics [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).

Numerous studies about liver galactose elimination capacity (GEC) [5, 9, 17, 22, 41, 78, 79, 82, 83, 88, 96]

Hepatic vein catheterization at high concentrations revealed a constant hepatic arterial-hepatic venous concentration difference [87,88].

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].

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).

Definition of velocity as amount of galactose removed per unit time per 100g of body weight (as opposed to rate per individual man [82, 96] or rate per g of liver weight [43, 50].

Blood flows unidirectionally through the sinusoids at prescribed rates [4, 26, 27]. The inflow concentration exceeds the outflow concentration with a gradient through the liver.

Apparent Km for galactose elimination in rat with sinusoidal perfusion model is 30.1 mcg/ml ~

0.167mM, which is in close agreement with values reported by others [18 27, 43, 48, 50];

The Vmax for galactose elimination is much higher in humans than in rats [47,82,96], with the Km being similar [47].

“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.

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

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

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 physic-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 [REF].

GEC in aging

“A significant negative correlation as observed between age and both liver volume and apparent liver blood flow. 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” {Wynne, 1989 #144}
“Schnegg1986{Schnegg, 1986 #145}
Wynne1989 {Wynne, 1989 #144}
Abnormalities in gal-Metabolism and liver disease: “Some patients with hepatic disease have increased Gal in their blood [Yamaguchi1989 -> 4], as shown by our patient with peliosis hepatitis, and also glycogen storage disease type XI. Also, liver dysfunction is an early clinical complication of galactosemia” [Yamaguchi1989]

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.

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 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.

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.

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.

Online Methods

The presented model of Human liver metabolism is a hierarchical model spanning cellular scale on the level of single hepatocytes (Figure 1A), tissue scale on level of the sinusoidal unit and the lobulus (Figure 1B) and organ scale via integration to the whole liver (Figure 1C).

Availability of data

The mathematical models on cellular scale and tissue-scale are provided as SBML under creative commons (CC BY-SA 4.0) in the supplementary information as well as on Biomodels.org and JWS Online. The complete source code of this project open source under GPL available from https://github.com/matthiaskoenig/multiscale-galactose. All datasets used for modelling are made accessible on request.

Cellular scale - galactose metabolism

The hepatocyte galactose metabolism is described by a kinetic model based on ordinary differential equations (ODEs). The model comprises the Leloir-Pathway with the main reactions GALK, GALT and GALE as well as the alternative processes important in galactosemias (Figure 1A). The ODEs are provided in the Supplementary Information with enzymatic parameters and metabolite concentrations listed in Supplementary Table 1 and Supplementary Table 2, respectively. The maximal enzyme activities (V_{max}) were chosen to achieve a good correspondence of model simulations with reported galactose elimination rates in healthy young human subjects Table 2 and Table 3 and observed changes in metabolite concentrations after galactose loads [REF, Figure?]. The kinetic parameters based on literature values were stored in SABIO-RK {Wittig2012} and are annotated in the SBML.

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. 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 (<200µm), namely galactose, water , albumin and sucrose, can pass in the space of Disse owing to the fenestration of the endothelial cells {Wisse1985}. Galactose and water can enter the hepatocytes, whereas sucrose and albumin are restricted to the space of Disse and the sinusoid. Diffusion and blood flow are modelled by discretizing the sinusoid and Disse space in small volumes with the transport between neighbouring volumes governed by one-dimensional diffusion and convection equations (analogue to {Konig2013}). The diffusion through the sinusoidal fenestration, small cylindrical channels in the endothelial cells between sinusoid and space of Disse are 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 by

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

The periportal (pp) and perivenous (pv) blood compartment 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.
All numerical integration of the ode systems were done with RoadRunner {REF} on a small computer cluster. Selected simulations were repeated with COPASI {Hoops2006} to check the results of the numeric integration. Simulation definitions and results were stored in a database.

Lobulus & Region of interests

To account for the heterogeneity of the sinusoidal units within the single liver lobules and the liver a distribution of sinusoidal units based on the reported values in blood flow v_{blood}, sinusoidal length L_{sin}, sinusoidal radius y_{sin}, width of Disse space y_{dis} and hepatocyte sheet thickness y_{cell} were simulated. The individual parameters were assumed statistically independent for the simulations.
Parameters for the log-normal distributions were fitted to experimental data based on a maximum-likelihood method for uni-variate distributions. The fit was performed with fitdistr from package MASS in R with estimate and estimate error given in (Supplementary Table 4)
For L_{sin} and y_{dis} no experimental histograms could be found in the literature, so the parameters were calculated from reported mean m and standard deviation std based on the transformation

$$std\log = \sqrt{\log(1 + \frac{std^2}{m^2})} \quad mean\log = \log(\frac{m^2}{\sqrt{m^2 + std^2}})$$

To simulate the model a sample of sinusoidal geometries and blood flows (N=1000 for dilution curves; N=250 for galactose simulations) was simulated.

Individualized liver

The scaling to the whole liver is performed based on prediction of the individual liver volume, liver blood flow and perfusion based on the anthropomorphic information of the subjects. For the prediction a multitude of GAMLSS {REF} models were fitted to describe the correlations between anthropomorphicc data and liver data. The information from multiple sources were integrated and a best prediction of the liver characteristics for the individual combination of age, gender, body weight, height and body surface area (BSA) performed.
Population variability was calculated

- GAMLSS data model fitting
- Combination of distributions for different antropomorphic features to get best estimate of liver blood flow and liver volume

Population variability

NHANES between years ? - ? was used. The data was filtered to bodyweight <24.9 and Caucasian. The combination of age, gender, height, weight and BSA.

BSA was calculated based on the formula by ? {}

Multiple indicator dilution curves

The area under the curve (AUC), mean transit time (MTT), and variance of the transit time (VTT) were calculated directly from the dilution curves using the following equations {Warren2008}:

$$AUC = \int_0^\infty s_{pp}^k(t) \cdot dt$$

$$MTT = \frac{\int_0^\infty t \cdot s_{pp}^k(t) \cdot dt}{AUC} \qquad VTT = \frac{\int_0^\infty t^2 \cdot s_{pp}^k(t) \cdot dt}{AUC} - (MTT)^2$$

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 dilution curves.

Galactose elimination and clearance

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_{pp}^{gal} and c_{pv}^{gal} using the following equations {Schirmer1986}

$$GE = (c_{pp}^{gal} - c_{pp}^{gal})$$

$$R = Q\,(c_{pp}^{gal} - c_{pp}^{gal})$$

$$ER = \frac{(c_{pp}^{gal} - c_{pp}^{gal})}{c_{pp}^{gal}}$$

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

Aging Simulations

The structural changes in microarchitecture in the liver in aging were simulated via age-dependent change in fenestration, i.e. change in fenestration number per area (N_{fen}) and endothelial thickness (y_{end}) based on experimental data (supplementary information)

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