A mathematical model of liver metabolism: from steady state to dynamic

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Abstract. The increase in Type 2 diabetes and other metabolic disorders has led to an intense focus on the areas of research related to metabolism. Because the liver is essential in regulating metabolite concentrations that maintain life, it is especially important to have good knowledge of the functions within this organ. In silico mathematical models that can adequately describe metabolite concentrations, flux and transport rates in the liver in vivo can be a useful predictive tool. Fully dynamic models, which contain expressions for Michaelis-Menten reaction kinetics can be utilized to investigate different metabolic states, for example exercise, fed or starved state. In this paper we describe a two compartment (blood and tissue) spatially lumped liver metabolism model. First, we use Bayesian Flux Balance Analysis (BFBA) to estimate the values of flux and transport rates at steady state, which agree closely with values from the literature. These values are then used to find a set of Michaelis-Menten parameters and initial concentrations which identify a dynamic model that can be used for exploring different metabolic states. In particular, we investigate the effect of doubling the concentration of lactate entering the system via the hepatic artery and portal vein. This change in lactate concentration forces the system to a new steady state, where glucose production is increased.

1. Introduction

The liver plays an integral part in the body’s metabolism, particularly in maintaining levels of glucose during fasting. When fasting, the glucose level in the blood drops, and the signal is given to the liver to begin manufacturing glucose, a process called gluconeogenesis. There are many substances that can be precursors to glucose, including glycogen stored in the liver, amino acids, and lactate. The present model, which describes a 24-hour fasted rat liver and is a variant of the one presented in [3], consists of two well-mixed compartments and the corresponding mass balance equations. Following the paradigm that a mathematical metabolism model should be able to capture the essential features of the in vivo organ while maintaining tractability, our model is based on the biochemical pathway shown in Figure 3, which describes the reactions that occur in the liver tissue. These reactions are somewhat simplified, with chains of reactions, e.g., the Citric Acid Cycle, represented as a single reaction. Furthermore, the tissue compartment is considered well-mixed and does not take into account the various sub-cellular structures, such
as cytosol and mitochondria, or the spatial heterogeneity of the liver [1] [4]. In summary, to reduce the complexity of the model, we consider the liver as a one-dimensional slice.

2. Model Description

Our liver model consists of a system of differential equations, referred to as dynamic mass balance equations, describing the changes in the concentration of different metabolites and intermediates in the blood and tissue compartments. Each species is accounted for separately in the two compartments, meaning that, for example, glucose in the tissue has a different mass balance equation than glucose in the blood. The 12 blood equations track the change of metabolite concentrations due to convection and transport between the blood and tissue compartments. More specifically, the dynamic mass balance equation for the concentration of species \( i \) in blood, \( 1 \leq i \leq 12 \), is

\[
\frac{dC^b_i}{dt} = \frac{F_b}{V_b} (C^*_i - C^b_i) - J^{b \rightarrow t}_i \left( \frac{V_t}{V_b} \right)
\]

(1)

where \( F_b \) is the blood flow rate through the liver, \( V_b \) and \( V_t \) are the blood and tissue volumes, respectively, \( J^{b \rightarrow t}_i \) is the transport from the blood to the tissue compartment, and \( C^*_i \) is the concentration of the metabolite in the blood upstream of the liver. The upstream blood is a mixture of oxygenated blood from the heart and nutrient-rich blood from the intestines.

The tissue compartment is a lumped representation of subcellular structures such as cytosol and mitochondria. In our model we track 27 biochemical species in tissue, which are produced or utilized by the biochemical reactions. Figure 3 displays the metabolic pathway in the liver, with the arrows representing some of the 28 reaction fluxes that we consider. The mass balances for each of the 27 metabolite and intermediate concentrations in the tissue account for the biochemical reactions producing or utilizing that species and for its transports into and from the blood compartment. The dynamic mass balance equation for the \( j \)th species, \( 1 \leq j \leq 27 \), is

\[
\frac{dC^t_j}{dt} = \sum_{k=1}^{28} s_{jk} \phi_k + \sum_{i=1}^{12} m_{ji} J^{b \rightarrow t}_i
\]

(2)

where \( s_{jk} \) is the stoichiometric coefficient indicating how many moles are consumed (\( s_{jk} < 0 \)) or produced (\( s_{jk} > 0 \)) by reaction \( \phi_k \), which denotes the reaction flux of reaction \( k \). The transport coefficient is defined \( m_{ji} = 1 \) if species \( j \) is transported between the blood and tissue by the transport \( J^{b \rightarrow t}_i \), \( m_{ji} = 0 \) otherwise.

2.1. Steady State Model and Bayesian Flux Balance Analysis

When the system is at steady state, the concentrations are assumed not to change in time, that is \( dC/dt = 0 \), hence the expressions for equations (1) and (2) can be substantially simplified. Letting \( \alpha = F_b/V_b \), \( \beta = V_t/V_b \), and introducing the vectors

\[
C^{*,b} = \begin{bmatrix}
(C^*_1 - C^b_1) \\
(C^*_2 - C^b_2) \\
\vdots \\
(C^*_12 - C^b_{12})
\end{bmatrix} \in \mathbb{R}^{12\times 1}, \quad J = \begin{bmatrix}
J^{b \rightarrow t}_1 \\
J^{b \rightarrow t}_2 \\
\vdots \\
J^{b \rightarrow t}_{12}
\end{bmatrix} \in \mathbb{R}^{12\times 1}, \quad \Phi = \begin{bmatrix}
\phi_1 \\
\phi_2 \\
\vdots \\
\phi_{28}
\end{bmatrix} \in \mathbb{R}^{28\times 1},
\]

we can write the blood and tissue equations at steady state in the form

\[
0 = \alpha IC^{*,b} - \beta IJ \quad b = S \Phi + MJ
\]
where $I \in \mathbb{R}^{12 \times 12}$ is the identity matrix, $0 \in \mathbb{R}^{12 \times 1}$ is a vector of zeros, $S = [s_{jk}] \in \mathbb{R}^{27 \times 28}$ is the stoichiometric matrix, $M = [m_{ji}] \in \mathbb{R}^{27 \times 12}$ is the transport matrix and the vector $b$ contains all zeros except for the row representing glycogen, which we assume is depleted at a constant rate.

Collecting the steady state equations for the tissue and blood we obtain the linear system

$$
\begin{bmatrix}
S & M \\
-\beta I & \alpha I
\end{bmatrix}
\begin{bmatrix}
\Phi \\
J \\
C^{*},b
\end{bmatrix}
=
\begin{bmatrix}
b \\
0
\end{bmatrix},
$$

(3)

Our first inverse problem is to estimate the unknowns in the vector $x$, which we solve by modeling the unknown vector $x$ as random variables and applying the recently proposed Bayesian Flux Balance Analysis (BFBA) methodology [5]. More precisely, we replace the linear system (3) with its stochastic extension

$$
Ax - r = e
$$

(4)

where the random variable $e$ represents error from modeling and uncertainty about the system being at steady state. Assuming that the probability distribution of $e$ is $\pi_{\text{model}}$, the Bayesian model for the steady state is given by

$$
\pi(e = 0|x) \propto \pi_{\text{model}}(Ax - r)
$$

(5)

where $\propto$ means proportional to. In our case, since the matrix $A$ is underdetermined, the linear system (3) does not admit a unique solution. In a Bayesian setting, however, we can use our a priori belief about the model and the unknowns to overcome this lack of data, namely the fact that:

- All of the reaction rates in $\Phi$ are positive and bounded from above;
- The transport rates in $J$ and concentration differences in $C^{*},b$ can be negative, but are bounded from above and below;
- The values of certain transports and reaction rates (i.e. the glycogen depletion rate) are approximately known.

We remark that the upper and lower bounds on our reaction rates and transports are rather loose and do not have a significant effect on the estimated values. In fact, the upper bound on the reaction fluxes and the bounds on the transports and concentration differences are introduced as a safeguard to guarantee that the density does not become improper. The transports and reaction rates that are assumed to be approximately known have values that are in agreement with those reported in the literature.

The prior information is encoded in the prior density $\pi_{pr}(x)$. If we interpret the model equation (4) as an observation, the density (5) can be thought of as the likelihood density, and, from Bayes formula, the posterior density for $x$ is

$$
\pi_{\text{post}}(x) \propto \pi_{\text{model}}(Ax - r)\pi_{pr}(x)
$$

(6)

We explore this posterior distribution using a Markov Chain Monte Carlo (MCMC) method which is a variant of the systematic-scan Gibbs sampler. The sampling is done sequentially, first by using a systematic-scan Gibbs to sample the space orthogonal to the null space of the matrix
A, and then using the Hit and Run algorithm to sample from the null space of A. For a more complete description of BFBA via MCMC methods, see [2].

Representative results from the MCMC simulation (sample size 100000 for reaction flux PEP→GAP) are shown in Figure 1. Notice the good mixing properties of the sample (left panel), and the Gaussian shape of the histogram (middle panel). The analysis of the auto-correlation functions (right panel) revealed good convergence properties for all the samples. We remark that the values determined by the model are in good agreement with those reported in the literature [3], plotted in black in the figure, confirming that our model captures well the liver in vivo during rested, fasting steady state.

Figure 1. Sample history of the reaction flux PEP→GAP, with the corresponding histogram and auto-correlation function of the sample. The black line is a value from the literature for this reaction flux at the fasted, resting steady state [3].

2.2. From Steady State to Dynamic

Once we have estimates of the reaction fluxes and transport rates at steady state, we can use them to determine the parameters for the kinetic model. If \( \phi_k \) is the flux of the facilitated reaction

\[
S + E \rightarrow P + \tilde{E}
\]

involving the single substrate \( S \), product \( P \), and facilitators \( E \) and \( \tilde{E} \), we express it in Michaelis-Menten form as

\[
\phi_k = V_{\text{max},k} \frac{C_S}{K_k + C_S \mu_k + R}
\]

where \( V_{\text{max},k} \) is the maximal initial velocity of this reaction, \( K_k \) and \( \mu_k \) are the reaction specific Michaelis-Menten constants, \( C_S \) is the concentration of substrate \( S \), and \( R = C_E/C_{\tilde{E}} \) is the ratio of the facilitators concentrations. If a single substrate reaction is not facilitated by an enzyme, the last term in the above expression is removed. Similarly, we express the flux of a facilitated bisubstrate reaction

\[
S_1 + S_2 + E \rightarrow P_1 + P_2 + \tilde{E},
\]

in the form:

\[
\phi_k = V_{\text{max},k} \frac{C_{S_1}C_{S_2}}{K_k + C_{S_1}C_{S_2} \mu_k + R}
\]

We denote by \( \Theta^1 = [[V_{\text{max},k}], [K_k], [\mu_k]] \in \mathbb{R}^{1 \times 83} \) the vector of all Michaelis-Menten parameters for the 28 reaction fluxes. There is one \( V_{\text{max}} \) per reaction, one \( K \) per reaction, and one \( \mu \) per facilitator, of which there are 27, yielding a total of 83 unknowns.
The transport of species \( j \) between blood and tissue, denoted by \( J_{b\rightarrow t}^j \), can be either passive or facilitated. In the case of bidirectional passive transport, the rate depends only on the scaled difference between the concentrations in the two compartments, hence we model it in the form

\[
J_{b\rightarrow t}^j = \lambda_i(C_b^j - \sigma_iC_t^j).
\]

Facilitated transport rates, on the other hand, are unidirectional and can be expressed in Michaelis-Menten forms analogous to those used for reaction fluxes, that is, either

\[
J_{b\rightarrow t}^j = W_{b}^i C_b^j M_{b}^i + C_b^j, \quad J_{t\rightarrow b}^j = W_{t}^i C_t^j M_{t}^i + C_t^j.
\]

We denote by \( \Theta^2 = [\lambda_i], [\sigma_i], [W_{b}^i], [M_{b}^i], [W_{t}^i], [M_{t}^i] \in \mathbb{R}^{1 \times 24} \) the vector containing each of the 2 Michaelis-Menten parameters for the 12 transport rates, resulting in 24 unknowns.

In order to fully identify a forward dynamic model, which is an initial value problem, we must specify the initial values. That is, we need to estimate the initial concentrations for the species in the blood \( C_b^i(0), 1 \leq i \leq 12 \), tissue \( C_t^j(0), 1 \leq j \leq 27 \) and the upstream concentrations \( C^*_i, 1 \leq i \leq 12 \), thus adding 51 unknowns which we collect in the vector \( \Theta^3 \in \mathbb{R}^{1 \times 51} \).

The second inverse problem of liver cellular metabolism amounts to estimating the vector of parameters for the reaction fluxes and transport rates, as well as the initial and upstream concentrations

\[
\Theta = [\Theta^1], [\Theta^2], [\Theta^3] \in \mathbb{R}^{1 \times 158}
\]

from the information gained via the Bayesian Flux Balance Analysis. From the posterior sampling of the densities given in equation 6, we have a collection of distribution estimates for the fluxes, transports, and arterial-venous differences contained in vector \( x \). Let us denote the mean of these distributions \( \bar{x} \). Let \( f(\Theta,t) \) be the function that maps to the vector of reaction fluxes, transport rates, and arterial-venous differences proposed by the dynamic model with parameter vector \( \Theta \) at time \( t \). The solution to the inverse problem are the posterior distributions that are given by the expression:

\[
\pi_{\text{post}}(\Theta) \propto \pi_{\text{likelihood}}(\bar{x} - f(\Theta,t))\pi_{\text{pr}}(\Theta)
\]

where \( \pi_{\text{pr}}(\Theta) \) is a prior on \( \Theta \) introducing loose bounds on the values. The solution of the inverse problem, which requires a combination of quasi-Newton optimization methods and sampling techniques, is beyond the scope of the paper and will not be discussed here. Once we have an estimate of \( \Theta \), or more generally, a probability distribution for it, it is possible to compute the predictive tissue \( C_t^j(t) \) and blood concentrations \( C_b^i(t) \) for \( 0 < t \leq t_{\text{max}} \) using our dynamic model.

3. Results

Using the kinetic model parameters and initial value concentrations \( \Theta \) corresponding to the MAP solution of the inverse problem, we want to investigate how the metabolic processes in the liver are affected by a change in the lactate concentration in blood. For this purpose we propagate the initial concentrations for 1500 minutes, then at \( t = 1500 \) we double the upstream concentration of lactate and propagate the concentrations in time for another 1500 minutes. Since it is known that lactate is a good precursor to glucose, we want to test if the predictions
of our kinetic model are in agreement with our qualitative expectation of an increase in the reaction fluxes involved in gluconeogenesis, and also in the the Citric Acid Cycle reaction which provides the extra energy demand of gluconeogenic reaction fluxes.

Figure 2 shows the comparison of the steady state flux values for three different reactions from the BFBA versus the flux values predicted by the forward dynamic model. The grey envelope marks the region within 1 standard deviation of the conditional mean of the steady state sample of BFBA, which is graphed as the black line in the center of the envelope. The thicker black curve for \( t < 1500 \), is the flux value predicted by the dynamic model for the original steady state. We remark that all except one of the flux values (glycogen flux) predicted by the dynamic model eventually settle within 1 standard deviation of the conditional mean of the steady state sample. The reason why the glycogen flux behaves differently is that the steady state analysis assumed the availability of a pool of glycogen which, in the long run of the dynamic simulation, is completely depleted.

![Figure 2](image)

**Figure 2.** The thick black curve displays values of glucose production (left), lactate uptake (middle), and Citric Acid cycle flux (right) in time. The thin black line is the conditional mean of the steady state flux values, and the grey envelope represents 1 standard deviation from the conditional mean. At \( t = 1500 \), the upstream lactate is doubled, resulting in a new steady state that has increased glucose production, lactate uptake, and Citric Acid cycle activity.

After the upstream lactate concentration is doubled at \( t = 1500 \), the dynamic model settled to a new, different steady state that is in fact in accordance with our qualitative expectations. At this new steady state, about 80% of the increased lactate is converted to glucose while the remaining 20% enters the Citric Acid cycle. Figure 3 shows the biochemical pathway in the tissue, with the net percentage differences between the new and the original steady state. This experiment illustrates the flexibility and stability of the dynamic model.

4. Conclusions and Future Work

Several experimental studies about liver metabolism deal with perfused (*ex vivo*) liver, for which it is easier to collect data. The model presented here is presently being modified to describe the perfused liver. Furthermore, because the liver is a highly heterogeneous organ, we plan to extend our model into a fully dynamic, spatially distributed one which can be used to test the effect of location on metabolic activity.

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Figure 3. Biochemical pathway of the liver, labeled with net changes in flux values after doubling the upstream lactate concentration.

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