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Dynamic Bayesian sensitivity analysis of a myocardial metabolic model

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Abstract

Dynamic compartmentalized metabolic models are identified by a large number of parameters, several of which are either non-physical or extremely difficult to measure. Typically, the available data and prior information is insufficient to fully identify the system. Since the models are used to predict the behavior of unobserved quantities, it is important to understand how sensitive the output of the system is to perturbations in the poorly identifiable parameters. Classically, it is the goal of sensitivity analysis to assess how much the output changes as a function of the parameters. In the case of dynamic models, the output is a function of time and therefore its sensitivity is a time dependent function. If the output is a differentiable function of the parameters, the sensitivity at one time instance can be computed from its partial derivatives with respect to the parameters. The time course of these partial derivatives describes how the sensitivity varies in time.

When the model is not uniquely identifiable, or if the solution of the parameter identification problem is known only approximately, we may have not one, but a distribution of possible parameter values. This is always the case when the parameter identification problem is solved in a statistical framework. In that setting, the proper way to perform sensitivity analysis is to not rely on the values of the sensitivity functions corresponding to a single model, but to consider the distributed nature of the sensitivity functions, inherited from the distribution of the vector of the model parameters.

In this paper we propose a methodology for analyzing the sensitivity of dynamic metabolic models which takes into account the variability of the sensitivity over time and across a sample drawn from the posterior density of the vector of the model parameters, viewed as a random variable. To interpret the output of this doubly varying sensitivity analysis, we propose visualization modalities particularly effective at displaying simultaneously variations over time and across a sample. We perform an analysis of the sensitivity of the concentrations of lactate and glycogen in cytosol, and of ATP, ADP, NAD⁺ and NADH in cytosol and mitochondria, to the parameters identifying a three compartment model for myocardial metabolism during ischemia.

Keywords

Myocardial metabolism; Bayesian statistics; Michaelis-Menten kinetics; sensitivity function; large scale sensitivity analysis; Markov Chain Monte Carlo

1 Introduction

Dynamic models of metabolic systems are identified by a large number of parameters, whose values are often very difficult to estimate due to the complexity of the model and the relative scarcity of the available data. As the complexity of the model increases, the number of parameters also grows, while the data available for their identification remains essentially the same. Thus the parameters identifying the more complex models are often known only approximately. When mathematical models are used to predict the behavior of a system under different initial conditions or with different inputs, it is important to know how much the output is affected by errors in the parameters, because this will suggest how reliable the predictions are. It is the purpose of sensitivity analysis to assess the extent of such dependency. The need for sensitivity analysis arises also in the context of model reduction and statistical estimation of the model parameters: in fact, if a quantity of interest is not sensitive to some of the model parameters, it is not crucial to estimate them accurately.

If the model is specified by the values of the parameter vector $\Theta = [\Theta_1, \dots, \Theta_n]$, and if the output function $C(\Theta)$ is a differentiable function of Θ , the sensitivity of $C(\Theta)$ to perturbations in Θ_j is traditionally expressed by the partial derivative

$$\frac{\partial C(\Theta)}{\partial \Theta_j}.$$

Since the value of this partial derivative depends on the values of $C(\Theta)$ and of Θ_j , and in metabolic systems the concentrations of the different metabolites can span several orders of magnitude, it is advisable to consider the relative change in the output following a relative change in the parameter value, that is, the dimensionless partial derivative

$$\frac{\partial C(\Theta)}{C} / \frac{\partial \Theta_j}{\Theta_j} = \frac{\Theta_j}{C(\Theta)} \cdot \frac{\partial C(\Theta)}{\partial \Theta_j},$$

which we will refer to as *sensitivity function* in the remainder of the paper.

The literature on sensitivity analysis for complex metabolic models is quite scarce, and it is usually restricted to models with a small number of components and identified by few parameters, usually 10 or fewer. Most of the sensitivity studies for larger models are performed at steady state, see [13,15]. Although the sensitivity analysis at steady state provides valuable information of the dependencies on the model parameters, an extrapolation to dynamic models may be misleading.

In a dynamic model, the output function, $C(\Theta) = C(\Theta, t)$, is a function of time, therefore the sensitivity functions are also time dependent. This means that the sensitivity of the output functions to perturbations in the parameters identifying the model may vary over time. Most of the dynamic sensitivity analyses for large scale complex models that have been proposed in the literature rely on simplifications of the sensitivity functions. The few which do not, require extensive computational resources. For example, the Metabolic Models Tools (MMT2) package in [6], which studies the time evolution of the sensitivity of complex biochemical networks, is based on the automatic differentiation package ADIFOR [5]. MMT2 proceeds by generating a multitude of models in the same basic family, specified by different parameter values, then eliminates those which are biologically meaningless. The MMT2 package can

handle models described by more than 10 ODEs, specified by over 100 parameters and large data sets (several thousand measured values). Its extensive computational needs are usually met by running it in a grid computing environment.

Our approach to sensitivity analysis differs from what has been previously proposed in the literature in several ways. In addition to not resorting to simplified models and to proposing a methodology which can be carried out on a personal computer, we advocate a sensitivity analysis which accounts for both the dynamic nature of the problem and the non identifiability of the model. The dynamic aspect of the sensitivity is addressed by computing the sensitivity functions, which we suggestively refer to as *dynamic sensitivity functions*, at several time instances. The evaluation of the dynamic sensitivity function, which is computationally much more expensive than when considering only the steady state case, clearly shows the variations in time of the sensitivity to the parameters. The statistical approach to the model identification problem proposed in [1] overcomes the non identifiability of the system by modeling the vector of model parameters Θ as a random variable, and by using its *distribution* to explain the observations and prior belief about the system. In the statistical framework, the sensitivity analysis also takes on a different form. Rather than proposing a single sensitivity function of questionable reliability, a whole distribution of sensitivity functions, corresponding to the distribution of the underlying parameters, is calculated. When the distribution of a sensitivity function is narrow and all feasible models exhibit essentially the same sensitivity, we conclude that the sensitivity is *stable* across a representative sample of parameters and therefore of models, hence the reliability of predictions based on the output can be easily assessed from any model realization in the sample. The reliability of predictions based on outputs whose sensitivity functions take on very different values for different sets of feasible parameters, on the other hand, may be hard to assess.

The paper is organized as follows. In Section 2 we motivate the Bayesian sensitivity analysis by showing its role in model reductions and in improving the convergence of MCMC sampling techniques. In Section 3 we review a compartmentalized model of myocardial metabolism and discuss the dependency of the system on various groups of parameter values. In Section 4 we apply our sensitivity analysis to the three compartment myocardial metabolism model outlined in Section 3. In particular, we study the sensitivity of the concentrations of glycogen and lactate in cytosol, and of ADP, ATP, NAD^+ and NADH in cytosol and mitochondria, to each one of the parameters specifying the model over an interval of time of 66 minutes during which we simulate moderate ischemia. We examine the stability of the sensitivity functions over a family of suitable parameter vectors which is perfectly in line with the Bayesian framework adopted for the parameter identification problem, where the solution, instead of being a single vector of parameter values which identify the model, is a probability density. A description of the model and detailed analysis of the results of the sensitivity analysis is given in the Appendix.

2 Sensitivity analysis: a Bayesian motivation

In this section, we discuss the key role of the proposed dynamic Bayesian sensitivity analysis when performing model reduction and in high dimensional parameter identification problems.

2.1 Model reduction

Assume that the data consist of few observed concentrations at a number of time instances. Modeling the fluctuation of the data, y , due to population variability and imprecisions in the measurements, with an additive random noise, e , we have the observation model

$$\mathbf{y}_i = \begin{bmatrix} C_{j_1}(\boldsymbol{\Theta}, t_i) \\ C_{j_2}(\boldsymbol{\Theta}, t_i) \\ \vdots \\ C_{j_m}(\boldsymbol{\Theta}, t_i) \end{bmatrix} + \mathbf{e}_i, \quad 1 \leq i \leq k,$$

or, denoting by P the sampling matrix that selects a few components $j_1; \dots j_m$ from the vector of concentrations,

$$\mathbf{y}_i = P\mathbf{C}(\boldsymbol{\Theta}, t_i) + \mathbf{e}_i. \quad (1)$$

The parameter estimation problem seeks to estimate the parameter $\boldsymbol{\Theta}$ or, in the statistical setting, its distribution from observations and any available prior information.

Typically optimization-based algorithms have great difficulties when the dimensionality of the parameter vector is high. Therefore it would be desirable to fix the values of some parameters to values taken e.g., from the literature, and to estimate only a subset of them. More precisely, partition the parameter vector into two parts, $\boldsymbol{\Theta} = [\boldsymbol{\Theta}', \boldsymbol{\Theta}']$, and fix $\boldsymbol{\Theta}'' = \boldsymbol{\Theta}_0''$ to some given values. The observation model can be expressed as

$$\begin{aligned} \mathbf{y}_i &= PC(\boldsymbol{\Theta}', \boldsymbol{\Theta}_0'', t_i) + \mathbf{e}_i \\ &= PC(\boldsymbol{\Theta}', \boldsymbol{\Theta}_0'', t_i) + P(\mathbf{C}(\boldsymbol{\Theta}', \boldsymbol{\Theta}'', t_i) - \mathbf{C}(\boldsymbol{\Theta}', \boldsymbol{\Theta}_0'', t_i)) + \mathbf{e}_i \\ &= PC(\boldsymbol{\Theta}', \boldsymbol{\Theta}_0'', t_i) + \mathbf{W}_i(\boldsymbol{\Theta}', \boldsymbol{\Theta}_0'', t_i) + \mathbf{e}_i. \end{aligned}$$

The vector $\mathbf{W}_i$, which we refer to as the *model reduction error*, represents the error introduced by reducing the number of free parameters. The proper Bayesian way to eliminate the parameter $\boldsymbol{\Theta}''$ from the model is to consider it as a *nuisance parameter* and marginalize over it. If an analytic expression for the posterior is available, it may be possible to carry out this marginalization analytically. This is typically not the case for complex models of metabolic systems, for which the posterior can only be explored via Markov Chain Monte Carlo sampling. An approximate marginalization procedure for model reduction is proposed in [8], where a sample of reduction error vectors is generated by drawing parameters from the prior density to estimate its distribution. This Bayesian model reduction, which does not depend on how $\boldsymbol{\Theta}$ has been partitioned, requires the marginalization procedure which may be computationally very expensive. Other approximations to the marginal likelihood have been proposed which utilize importance sampling of the posterior, see [4]. This method of approximation requires the careful selection of an importance sampling function, see [4,12], which if not chosen carefully, can lead to slow convergence.

Often, to avoid the estimation of the distribution of the reduction error, the model reduction is performed by fixing some of the parameter values and simply ignoring the model reduction error. In order for not producing a significant and uncontrolled error in the model, the parameter vector should be partitioned so that the neglected reduction error is not dominant compared to the error $\mathbf{e}_i$. This partitioning, however, requires that we know which components of $\boldsymbol{\Theta}$ have no or little effect on the data. The sensitivity analysis that we propose in this paper seeks to identify these components.

Traditionally, the sensitivity of the data to a given parameter is measured by its derivative with respect to that parameter. In the Bayesian setting, the data has no sensitivity to a particular group of parameters $\boldsymbol{\Theta}''$, if the derivatives with respect to all components of $\boldsymbol{\Theta}''$ are negligible over a sample, so that the reduction error is small as a random variable. We refer to the consistency of the derivative values over a sample as *stability of the sensitivity*.

2.2 Bayesian parameter estimation and output prediction

In the Bayesian framework, where the parameter estimation is based on Markov Chain Monte Carlo (MCMC) techniques, the convergence of a sample is an important issue. To obtain a reliable estimate for the conditional mean of a component of the parameter vector Θ , enough independent samples have to be collected from its posterior distribution. The Central Limit Theorem states that the asymptotic convergence rate for an average of independent and identically distributed random variables is $1/\sqrt{N}$, where N is the number of the variables. However, MCMC strategies such as the Metropolis-Hastings algorithm (see, e.g., [8,11]) do not produce a chain of independent samples. In the chain, each component of the vector has a different correlation length, and therefore we cannot expect the convergence rate to be the same. If k_j is the correlation length in the chain of the j th component, the convergence rate is approximately $1/\sqrt{N/k_j}$. Since the correlation lengths k_j may be large for some of the components, a reliable estimation of the distributions of all the parameters may be computationally unfeasible.

In the study of metabolic models, we are not usually interested in the model parameters, but in the *predicted time course* of certain metabolites or facilitators under given conditions. The reliability of the output prediction depends on how well we are able to estimate the parameters of the model. In this context, the sensitivity analysis may play a crucial role: it is unnecessary to run a huge MCMC sample to estimate accurately a parameter that has no effect on the relevant quantities.

3 Model identification

The three compartment model to which we apply our analysis, described in [1], is a modification of the one proposed in [16]. The dynamics of the species concentrations are described by a system of 46 nonlinear stiff ordinary differential equations which depend on 147 parameters. To define our model for cardiac metabolism, we assign a model parameter vector with 147 components of the form

$$\mathbf{x} = [\mathbf{C}_0, \mathbf{V}_{\max}, \mathbf{K}, \mu, \nu, \mathbf{T}, \mathbf{M}, \lambda, \sigma] = [\mathbf{C}_0, \Theta] \in \mathbb{R}^{147},$$

where $\mathbf{C}_0$ contains the initial values of the species concentrations. We assume that the initial state is a steady state. The meaning of the model parameters Θ is explained below, as we specify the kinetic expressions for the reaction fluxes and transport rates.

The system of differential equations expressing the change of the species in compartment ℓ has the general form

$$V_\ell \frac{d\mathbf{C}_\ell(\Theta)}{dt} = \underbrace{\mathbf{R}_\ell}_{\text{Reaction}} + \underbrace{\mathbf{K}_\ell}_{\text{Convection}} + \underbrace{\sum_k \mathbf{T}_{\ell k}}_{\text{Transport}}, \quad k, \ell = \text{b, c} \quad \text{or} \quad \text{m} \quad (2)$$

where $\mathbf{C}_\ell$ is the vector of the concentrations of all species in compartment ℓ and V_ℓ is the effective volume. The compartments are the blood (b), cytosol (c) and mitochondria (m). Since no reactions occur in the blood, the reaction term, $\mathbf{R}_\text{b}$, is zero. Since only the blood carries biochemical compounds from the outside, there is no convection in the cytosol and mitochondria. In the blood domain, we assume that the convection is proportional to the difference of the arterial and venous concentrations in the blood,

$$\mathbf{K}_\text{b} = \frac{Q(t)}{F} (\mathbf{C}_\text{a} - \mathbf{C}_\text{b}),$$

where $Q(t)$ is the blood flow, F is the mixing factor and C_a is the vector containing the arterial concentrations. These input functions are all assumed known.

The transport term $\mathbf{T}_{\ell k}$ is the rate of transport of species into the compartment ℓ from compartment k minus the rate of transport in the other direction. Not all biochemical compounds are transported between all compartments, and the transport may be occurring only in one direction. For example, if the species j is transported into the mitochondria from the cytosol and vice versa, the transport term in mitochondria is of the form

$$(\mathbf{T}_{mc})_j = J_{c \rightarrow m, j} - J_{m \rightarrow c, j}, \quad (3)$$

where $J_{m \rightarrow c, j}$ and $J_{c \rightarrow m, j}$ are the transport rates from mitochondria to cytosol and from cytosol to mitochondria respectively. Here, either one or both of the fluxes may vanish.

A non-vanishing transport may be passive, i.e., diffusion driven, in which case we model it as

$$J_{k \rightarrow \ell, j} = J_{k \rightarrow \ell, j}^p = \lambda_{k \rightarrow \ell, j} (C_{k, j} - \sigma_{k \rightarrow \ell, j} C_{\ell, j}), \quad (4)$$

where $\lambda_{k \rightarrow \ell, j}$ is the membrane transport coefficient and $\sigma_{k \rightarrow \ell, j}$ is the affinity coefficient, or carrier facilitated, in which case we describe it using the Michaelis-Menten form

$$J_{k \rightarrow \ell, j} = J_{k \rightarrow \ell, j}^f = T_{k \rightarrow \ell, j} \frac{C_{k, j}}{M_{k \rightarrow \ell, j} + C_{k, j}}, \quad (5)$$

where $T_{k \rightarrow \ell, j}$ is the maximum transport rate and $M_{k \rightarrow \ell, j}$ is the affinity coefficient. The parameters λ , σ , T and M , with appropriate subindices, are the components of the model parameter vectors λ , σ , T and M , respectively. The transport fluxes included in our model and their type (passive/facilitated) are listed in Table 14 in the Appendix.

The reaction term is a linear combination of reaction fluxes, with the linear coefficients assigned by the stoichiometric matrix S

$$(\mathbf{R}_{\ell})_j = \sum s_{jk}^{\ell} \Phi_k^{\ell}.$$

Here, Φ_k^{ℓ} is the reaction flux of the k th reaction in compartment ℓ and the coefficient s_{jk}^{ℓ} is positive if the compound j is produced in the reaction, negative if it is consumed and zero otherwise.

The reaction fluxes are assumed to be of Michaelis-Menten form. For example, the flux of a single substrate reaction coupled to a metabolic controller, $S + E \rightarrow \tilde{S} + \tilde{E}$, is expressed as

$$\Phi = V_{\max} \frac{C_E / C_{\tilde{E}}}{\beta + C_E / C_{\tilde{E}}} \frac{C_S}{K + C_S}, \quad \beta = v, \mu \quad (6)$$

where E , $\tilde{E}$ are regulatory species (e.g., ATP, ADP, in which case we use the symbol $\beta = \mu$ for the affinity constant, or NADH, NAD^+ , in which case $\beta = v$ is used), S is the substrate and the maximum velocity, $V_{\max}$, and the affinity coefficients, K , μ and v are reaction-specific. For bi-substrate reactions, $S_1 + S_2 + E \rightarrow \tilde{S}_1 + \tilde{S}_2 + \tilde{E}$, the reaction flux is expressed as

$$\Phi = V_{\max} \frac{C_E / C_{\tilde{E}}}{\beta + C_E / C_{\tilde{E}}} \frac{C_{S_1} C_{S_2}}{K + C_{S_1} C_{S_2}}, \quad \beta = v, \mu \quad (7)$$

Finally, if no regulatory species are involved, we set $\beta = 0$, which automatically sets the regulatory factor equal to one. The model parameters associated with the reaction terms constitute the components of the model vectors $\mathbf{V}_{\max}$, $\mathbf{K}$, $\boldsymbol{\mu}$ and $\mathbf{v}$, respectively. The list of the

reactions included in our model is given in the Appendix, Table 12. The stoichiometric coefficients can be read off from Table 13.

In [1] we assume that the observed concentrations of the metabolites used for the solution of the parameter estimation problem are distributed according to a probability distribution reflecting the uncertainties and measurement errors in the data, see [14], and that at different time instances the measured concentrations are conditionally independent. That is, we assume an additive error model,

$$\mathbf{y}_j = f(\mathbf{x}, t_j) + \mathbf{e}_j \in \mathbb{R}^2 \quad \text{for } 1 \leq j \leq 4 \quad (8)$$

where $\mathbf{e}_j$ is a zero mean Gaussian random variable with mutually independent components and variances estimated from [14]. The parameter estimation problem, which we solve in a Bayesian setting in [1], is to infer on the initial values C_0 and the model parameters Θ from the few measured quantities $\mathbf{y}$ taken during the ischemia episode.

In [1], measurements of the concentration of glycogen and lactate in the cytosol at few time instances during ischemia were used in the solution of the parameter estimation problem, which was severely underdetermined and ill-posed, thus requiring a combination of numerical and statistical techniques with the additional information that the system was at steady state before the onset of ischemia, to lead into a meaningful solution. The posterior density for the parameter vector was explored by producing a sample of 15000 parameter vectors generated with an adapted Metropolis-Hastings algorithm: see [1] for details. It is this sample that we utilize for our sensitivity analysis.

4 Time propagation of sensitivity functions

Our sensitivity analysis is concerned with determining the extent to which the estimated concentrations of the different biochemical species are affected by perturbations in the components of the vector $\Theta \in \mathbb{R}^n$ which contains the model parameters. More precisely, since the scaled partial derivatives

$$\frac{\Theta_j}{C_k(\Theta, t)} \frac{\partial C_k(\Theta, t)}{\partial \Theta_j} = \frac{\partial \log C_k(\Theta, t)}{\partial \log \Theta_j}, \quad (9)$$

which are our sensitivity functions, express the instantaneous rate of relative change of the concentration C_k at time t with respect to Θ_j , their absolute values indicate the sensitivity of C_k to perturbations in Θ_j at time t . Due to the dynamic nature of the model, it is possible that a concentration which is very sensitive to a parameter in some time subinterval, becomes essentially insensitive to it in a different one, therefore suggesting the necessity of studying the sensitivity over time.

The time dependent nature of the sensitivity functions implies that the derivatives of the concentrations with respect to the parameters need to be computed at different time instances. For our model, the analytic computation of the partial derivatives of the concentrations with respect to the parameters of the model is quite complex, because the reaction fluxes depend on the concentrations, and hence implicit differentiation formulas are needed. Since the complexity of the analytic formulas for the sensitivity functions would not lessen the computational burden, in our computed examples we use a numerical approximation for partial derivatives. More precisely, we use the finite difference approximations

$$\frac{\log \partial C_k}{\partial \log \Theta_j}(\Theta, t) \approx \frac{1}{\delta} \left(\frac{C_k(\Theta_j, t)}{C_k(\Theta, t)} - 1 \right), \quad 1 \leq k \leq m, \quad 1 \leq j \leq n, \quad (10)$$

where $C_k(\Theta, t)$ and $C_k(\Theta_j, t)$ are the values of the concentration C_k determined by the models specified by the parameter vectors Θ and $\Theta_j = [\Theta_1, \Theta_2, \dots, \Theta_j \exp(1+\delta), \dots, \Theta_n]$ at time t . This defines a matrix valued mapping,

$$\mathbb{R}^n \times \mathbb{R} \rightarrow \mathbb{R}^{m \times n}, \quad (\Theta, t) \mapsto D_{\log \Theta}(\log C)(\Theta, t) = \left[\frac{\partial \log C_k}{\partial \log \Theta_j}(\Theta, t) \right] \approx \left[\frac{1}{\delta} \left(\frac{C_k(\Theta_j, t)}{C_k(\Theta, t)} - 1 \right) \right],$$

which we refer to as the *sensitivity matrix*. We remark that to compute $C_k(\Theta, t_i)$ and $C_k(\Theta_j, t_i)$ we need to solve (2) twice, once for Θ and once for Θ_j . However, since $C(\Theta, t_i)$ does not depend on the perturbed component, it need to be computed only once. Thus, the computation of the sensitivity matrix at one value Θ of the parameter vector requires the solution of (2) $n + 1$ times.

To show that the sensitivity matrix depends on the parameter vector, Θ , where it is evaluated, we compute the sensitivities of the concentration of NADH in cytosol and mitochondria to all the $V_{\max}$ and K parameters with models corresponding to two different parameter vectors: Θ_0 , which was used in [1] as a starting point for the MCMC iteration and Θ_{CM} , the conditional mean estimate computed from the MCMC sample. Since we are only concerned with the sensitivity to the first 50 parameters at the $t = 1, 5, 12, 20, 30, 50, 65$ minutes after the beginning of ischemia simulation, we evaluate the four 7×50 matrices:

$$\left[\frac{\Theta_j}{C_{\text{NADH,c}}(\Theta)} \cdot \frac{\partial C_{\text{NADH,c}}(\Theta, t_i)}{\partial \Theta_j} \right] \quad \text{and} \quad \left[\frac{\Theta_j}{C_{\text{NADH,m}}(\Theta)} \cdot \frac{\partial C_{\text{NADH,m}}(\Theta, t_i)}{\partial \Theta_j} \right], \quad 1 \leq i \leq 7, \quad 1 \leq j \leq 50,$$

where

$$\Theta \in \{\Theta_0, \Theta_{\text{CM}}\}.$$

The absolute values of these matrices are displayed as pixel intensities in Figure 1 with appropriately chosen color scales. The color bars on the right of the images indicate the absolute values of the pixels of the images. The vertical axis represents time and the horizontal axis the components of the vector $[V_{\max}, K]$.

The first observation is that the sensitivities show a clear change of behavior in time, thus suggesting that extrapolation of the steady state sensitivities might not correctly identify sensitive parameters during ischemia. The second observation is that the sensitivities depend on the parameter vector Θ , thus indicating the limitations of a sensitivity analysis performed on a single parameter.

Following the fundamental paradigm of statistical solutions of inverse problems, we explain here how to perform sensitivity analysis respecting the random nature of the parameter vector and explore its stability using the sample. In the Bayesian setting used in [1] for the estimation of the parameters which specify the model, we explored the posterior through a Metropolis-Hastings sampling scheme, and collected a sample of 15000 parameter vectors which we use in our computed examples. To verify the stability of the sensitivity functions across the posterior sample, we evaluate the sensitivity functions at various realizations of the parameter vector. This additional analysis may give insight into the locality of the sensitivity results in the parameter space. Due to the computationally intensive nature of the evaluation process, we only consider a subsample of size 500. To diminish the correlation between realizations, the Markov chain is subsampled by picking vectors at a fixed interval apart from each other.

In Figure 2 we show the histograms and confidence envelopes for the sensitivity functions of the glycogen concentration in cytosol to $V_{\text{ATP} \rightarrow \text{ADP}}$ and of NAD^+ in cytosol to the initial concentration of bADP,

$$\left[\frac{V_{\text{ATP} \rightarrow \text{ADP}}}{C_{\text{GLY,c}}(\Theta)} \cdot \frac{\partial C_{\text{GLY,c}}(\Theta, t_i)}{\partial V_{\text{ATP} \rightarrow \text{ADP}}} \right] \quad \text{and} \quad \left[\frac{C_{\text{o,bADP}}}{C_{\text{NAD}^+, \text{m}}(\Theta)} \cdot \frac{\partial C_{\text{NAD}^+, \text{m}}(\Theta, t_i)}{\partial C_{\text{o,bADP}}} \right],$$

at seven different time instances: $t = 1, 5, 12, 20, 30, 50, 65$, over the mentioned subsample.

The plots illustrate that the behavior of the sensitivity functions may vary across the sample as well as in time. In particular, the sensitivity of NAD^+ to $V_{\text{ATP} \rightarrow \text{ADP}}$ is high at the onset of ischemia and decreases over time. The large width of the confidence envelope indicates that there is high variability across the sample, therefore suggesting that the sensitivity to this parameter computed at the conditional mean might not be statistically representative. The concentration of glycogen computed by the model gradually becomes more sensitive to perturbations in the value of initial bADP concentrations starting 12 minutes from the onset of ischemia until the end of the experiment, where the sensitivity is very high. The little variability in the sensitivity results across the sample indicates that the sensitivity calculated, e.g., at Θ_{CM} is representative for the whole sample.

5 Discussion of results

After a preliminary assessment of the methodology, we discuss the results of the analysis in a more systematic way. A full list and a sensitivity classification of the 147 parameters that describe the model is given in the Appendix. The discussion in this section will focus on sensitivity analysis of a few concentrations of interest, that is, those measured in [16], i.e., cytosolic glycogen and lactate, as well as of the concentrations of the key facilitators ATP, ADP, NAD^+ and NADH in both cytosol and mitochondria. We remark that a similar analysis can be carried out for any concentration of interest.

5.1 Sensitivities by group

The sensitivity coefficients are categorized according to maximal magnitude of their absolute value at the seven time instances considered. We classify the sensitivity as: high (H) for values greater than 1, moderate (M) for values between .1 and 1, low (L) for values between .05 and .1, and not sensitive (NS) for values less than .05. The detailed classification is presented in Table 1–Table 11 in the Appendix. The results partitioned by parameter types are presented below.

Sensitivity to V_{max} —Glycogen and lactate are moderately to highly sensitive to the V_{max} parameters associated with the ATP hydrolysis ($\text{ATP} \rightarrow \text{ADP}$) and ADP conversion ($\text{ATP} \leftrightarrow \text{bADP}$). These concentrations are also sensitive to the V_{max} parameters associated with glycogen utilization ($\text{GLY} \rightarrow \text{G6P}$), oxidative phosphorylation ($\text{O}_2 \rightarrow \text{H}_2\text{O}$) and pyruvate reduction ($\text{PYR} \rightarrow \text{LAC}$). Of the metabolic controllers, cytosolic NAD^+ and NADH are the most sensitive to the V_{max} parameters.

Sensitivity to K —The concentrations of interest show a similar sensitivity to the K parameters as to the V_{max} parameters. For example, the lactate and glycogen concentrations are most sensitive to changes in $K_{\text{G6P} \rightarrow \text{GLY}}$, $K_{\text{PYR} \rightarrow \text{LAC}}$, $K_{\text{FFA} \rightarrow \text{FAC}}$, $K_{\text{ADP} \leftrightarrow \text{bADP}}$, $K_{\text{ATP} \rightarrow \text{ADP}}$ and $K_{\text{OAA} \rightarrow \text{CIT}}$. The facilitators in the cytosol show strong sensitivity to the K parameters associated with ATP hydrolysis and ADP conversion. The metabolic controllers NAD^+ and NADH are sensitive to changes in the K parameters corresponding to reactions in the mitochondria, including the TCA cycle and oxidative phosphorylation.

Sensitivity to the μ and v parameters—Both lactate and glycogen are sensitive to perturbations in $\mu_{\text{GLY} \rightarrow \text{G6P}}$. Lactate is also moderately sensitive to the $v_{\text{G6P} \rightarrow \text{GLY}}$ and $v_{\text{O}_2 \rightarrow \text{H}_2\text{O}}$. Cytosolic NAD^+ and NADH are most sensitive to $\mu_{\text{GLY} \rightarrow \text{G6P}}$ and the v associated with the bidirectional reaction $\text{PYR} \leftrightarrow \text{LAC}$. The concentration of NAD^+ in both cytosol and mitochondria are sensitive to $v_{\text{MAL} \rightarrow \text{OAA}}$ and $v_{\text{O}_2 \rightarrow \text{H}_2\text{O}}$.

Sensitivity to the initial concentrations—The concentration of NAD^+ and NADH in the mitochondria, and of ATP and ADP in both mitochondria and cytosol are nearly insensitive to the cytosolic and blood concentrations. The concentrations of cytosolic NAD^+ and NADH are most sensitive to concentrations of GLU and GLY in the cytosol, O_2 in the blood and those concentrations corresponding to species in the TCA cycle.

Sensitivity to $\mathbf{T}$ and $\mathbf{M}$ —The lactate concentration is sensitive to perturbations of all the $\mathbf{T}$ and $\mathbf{M}$ parameters except for those corresponding to the transport of glucose. Glycogen is highly sensitive to the $\mathbf{T}$ and $\mathbf{M}$ parameters for the transport of P_i , ATP and ADP . The concentrations of interest are sensitive to the $\mathbf{T}$ and $\mathbf{M}$ parameters that describe the transport of enzymes facilitators: NADH , NAD^+ , ADP and ATP , between cytosol and mitochondria. This is not surprising since these species play an important role in the enzyme facilitated metabolic reactions in the cellular domain. The transport of NAD^+ from mitochondria to cytosol and the transport of NADH from cytosol to mitochondria, describes a simplified model of the malate-aspartate shuttle, see [10] for details.

Sensitivity to the λ and σ —The concentrations of interest are most sensitive to the λ and σ for the transport of FFA and O_2 .

5.2 Sensitivity at a glance

In addition to the tabular representation of the sensitivities of selected concentrations to the model parameters, we present a more suggestive way of visualizing the results. The time course of each concentration depends on the parameters via reaction and transport fluxes, which in turn are effectively visualized in pathway charts, see Figure 3–Figure 4. The pathway charts are color coded according to the sensitivity of each reaction and transport to the parameters of their kinetic expressions. The individual model parameters to which the reaction or transport fluxes are most sensitive are noted adjacent to the pathway in the color corresponding to the sensitivity level.

The chart of the sensitivity of the concentrations of glycogen and lactate is displayed in Figure 3. The pathways are classified according to the most sensitive parameter, for example, of glucose utilization ($\text{GLU} \rightarrow \text{G6P}$) reaction depends on the parameters, $\mathbf{V}_{\max}$, $\mathbf{K}$ and μ and on the concentration of glucose, ATP and ADP . This reaction is classified as moderately sensitive, since the concentrations of interest are moderately sensitive to the $\mathbf{V}_{\max}$ and μ parameters, whereas little or not sensitive to the remaining parameters. This reaction is color coded as moderately sensitivity (purple) and the *dominant* parameters responsible for this sensitivity level, $\mathbf{V}_{\max}$ and μ , are indicated with the same color code.

6 Conclusions and future work

In this paper we show that the sensitivity of the concentrations of biochemical species in a complex dynamic metabolic model for myocardial metabolism to the parameters which specify the model can vary significantly over time and across the distribution of suitable parameters. In addition, we propose a visualization methodology which can be quite effective for assessing the importance, in time and across the parameter space, of the various parameters identifying the model. A detailed analysis of the sensitivity of concentrations of key facilitators and cytosolic glycogen and lactate concentrations based at the conditional mean of the parameter vector is also presented. Analogous sensitivity studies can be performed at any point in parameter space.

Future work will be concerned with sensitivity guided model reductions for complex dynamic models of metabolic systems.

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Appendix

7 Appendix

In this appendix, we present the detailed sensitivity analysis of the concentrations of glycogen, lactate, and the metabolic regulatory species NAD^+ , NADH , ADP and ATP in the cytosol and mitochondria, to the 147 parameters that describe our model at the conditional mean. The sensitivity is classified according to the magnitude of the sensitivity coefficients with respect to time. The 147 parameters are partitioned into the groups: $V_{\max}$, K , μ , v , C_c , C_m , C_b , T , M , λ and σ , and the sensitivity analysis is presented in Table 1–Table 11.

In the cytosol domain, 21 mass balance equations specify the dynamics of the concentrations, 10 of these occur in the glycogenic sub-domain. The dynamics of the concentrations in the mitochondria and in the blood are modelled with 19 and 6 mass balance equations respectively. While the equations are not given here explicitly, we list the transport, metabolic reactions, fluxes and reactions rates which specify them are given in Table 12–Table 14.

Table 1

Overall sensitivity to the $V_{\max}$ parameters: H: high, M: moderate, L: low, NS: not sensitive.

$V_{\max}$	GLY	LAC	NAD^+_c	NADH_c	ADP_c	ATP_c	NAD^+_m	NADH_m	ADP_m	ATP_m
$V_{\text{GLU} \rightarrow \text{G6P}}$	NS	NS	M	M	NS	NS	NS	NS	NS	NS
$V_{\text{G6P} \rightarrow \text{GLY}}$	M	NS	L	L	NS	NS	NS	NS	NS	NS
$V_{\text{GLY} \rightarrow \text{G6P}}$	H	M	M	M	NS	NS	NS	NS	NS	NS
$V_{\text{G6P} \rightarrow \text{GAP}}$	NS	M	M	M	NS	NS	NS	NS	NS	NS
$V_{\text{GAP} \rightarrow \text{BPG}}$	NS	NS	M	NS	NS	NS	NS	NS	NS	NS
$V_{\text{BPG} \rightarrow \text{PYR}}$	NS	L	L	M	NS	NS	NS	NS	NS	NS
$V_{\text{PYR} \rightarrow \text{LAC}}$	L	M	M	M	NS	L	NS	NS	NS	NS
$V_{\text{LAC} \rightarrow \text{PYR}}$	NS	NS	L	M	NS	NS	NS	NS	NS	NS
$V_{\text{TG} \rightarrow \text{GLR}}$	NS	NS	M	L	NS	NS	NS	NS	NS	NS
$V_{\text{GLR} \rightarrow \text{TG}}$	NS	NS	M	L	NS	NS	NS	NS	NS	NS
$V_{\text{FAC} \rightarrow \text{FAC}}$	NS	M	H	M	NS	NS	M	M	NS	NS
$V_{\text{PCF} \rightarrow \text{CF}}$	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
$V_{\text{CF} \rightarrow \text{PCF}}$	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
$V_{\text{ATP} \rightarrow \text{ADP}}$	M	M	M	M	NS	H	NS	NS	NS	NS
$V_{\text{ADP} \rightarrow \text{bADP}}$	H	M	H	M	H	L	NS	NS	NS	NS
$V_{\text{bADP} \rightarrow \text{ADP}}$	H	M	H	M	H	L	NS	NS	NS	NS
$V_{\text{FAC} \rightarrow \text{ACoA}}$	NS	NS	M	M	NS	NS	NS	NS	NS	NS
$V_{\text{PYR} \rightarrow \text{ACoA}}$	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
$V_{\text{OAA} \rightarrow \text{CIT}}$	NS	M	M	M	NS	NS	M	M	NS	NS
$V_{\text{CIT} \rightarrow \text{KG}}$	NS	NS	M	M	NS	NS	M	NS	NS	L
$V_{\text{KG} \rightarrow \text{SCA}}$	NS	NS	NS	NS	NS	NS	L	NS	NS	NS
$V_{\text{SCA} \rightarrow \text{SUC}}$	NS	NS	L	NS	NS	NS	NS	NS	NS	NS
$V_{\text{SUC} \rightarrow \text{MAL}}$	NS	L	M	L	NS	NS	M	NS	NS	NS
$V_{\text{MAL} \rightarrow \text{OAA}}$	NS	L	M	M	NS	NS	M	NS	NS	NS
$V_{\text{O}_2 \rightarrow \text{H}_2\text{O}}$	M	L	M	M	NS	M	M	M	M	M

Table 2

Overall sensitivity to the K parameters: H: high, M: moderate, L: low, NS: not sensitive.

K	GLY	LAC	NAD^+_c	NADH_c	ADP_c	ATP_c	NAD^+_m	NADH_m	ADP_m	ATP_m
$K_{\text{GLU} \rightarrow \text{G6P}}$	NS	NS	M	M	NS	NS	NS	NS	NS	NS
$K_{\text{G6P} \rightarrow \text{GLY}}$	NS	NS	M	NS	NS	NS	NS	NS	NS	NS
$K_{\text{GLY} \rightarrow \text{G6P}}$	H	L	M	M	NS	NS	NS	NS	NS	NS
$K_{\text{G6P} \rightarrow \text{GAP}}$	NS	L	L	M	NS	NS	NS	NS	NS	NS
$K_{\text{GAP} \rightarrow \text{BPG}}$	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

K	GLY	LAC	NAD ⁺ _c	NADH _c	ADP _c	ATP _c	NAD ⁺ _m	NADH _m	ADP _m	ATP _m
K _{BPg} → PYR	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
K _{PYR} → LAC	NS	M	M	M	NS	L	NS	NS	NS	NS
K _{LAC} → PYR	NS	NS	L	L	NS	NS	NS	NS	NS	NS
K _{TG} → GLR	NS	NS	L	NS	NS	NS	NS	NS	NS	NS
K _{GLR} → TG	NS	NS	L	NS	NS	NS	NS	NS	NS	NS
K _{FEA} → EAC	NS	L	M	M	NS	NS	M	M	NS	NS
K _{PCr} → Cr	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
K _{Cr} → PCr	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
K _{ATP} → ADP	H	M	M	M	NS	H	NS	NS	NS	NS
K _{ADP} → ADP	H	M	M	M	M	NS	NS	NS	NS	NS
K _{ADP} → ADP	H	M	M	H	H	L	NS	NS	NS	NS
K _{EAC} → AC _{CoA}	NS	M	L	M	NS	NS	NS	NS	NS	NS
K _{PYR} → AC _{CoA}	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
K _{OAA} → CIT	NS	M	M	M	NS	NS	M	M	NS	NS
K _{CIT} → α-KG	NS	NS	L	L	NS	L	M	L	NS	L
K _{α-KG} → SCA	L	NS	L	NS	NS	NS	NS	NS	NS	NS
K _{SCA} → SUC	NS	NS	L	NS	NS	NS	NS	NS	NS	NS
K _{SUC} → MAL	NS	NS	M	L	NS	NS	M	NS	NS	NS
K _{MAL} → OAA	NS	NS	M	L	NS	NS	M	NS	NS	NS
K _{O₂} → H ₂ O	NS	L	M	M	NS	M	M	M	M	M

Table 3

Overall sensitivity to the μ parameters: H: high, M: moderate, L: low, NS: not sensitive.

μ	GLY	LAC	NAD ⁺ _c	NADH _c	ADP _c	ATP _c	NAD ⁺ _m	NADH _m	ADP _m	ATP _m
$\mu_{GLU \rightarrow G6P}$	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
$\mu_{G6P \rightarrow GLY}$	M	NS	NS	NS	NS	NS	NS	NS	NS	NS
$\mu_{GLY \rightarrow G6P}$	H	M	M	M	NS	NS	NS	NS	NS	NS
$\mu_{G6P \rightarrow GAP}$	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
$\mu_{BPg \rightarrow PYR}$	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
$\mu_{FEA \rightarrow TG}$	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
$\mu_{FEA \rightarrow EAC}$	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
$\mu_{PCr \rightarrow Cr}$	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
$\mu_{Cr \rightarrow PCr}$	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
$\mu_{SCA \rightarrow SUC}$	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
$\mu_{O_2 \rightarrow H_2O}$	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Table 4

Overall sensitivity to the v parameters: H: high, M: moderate, L: low, NS: not sensitive.

v	GLY	LAC	NAD ⁺ _c	NADH _c	ADP _c	ATP _c	NAD ⁺ _m	NADH _m	ADP _m	ATP _m
$v_{GAP \rightarrow BPg}$	NS	NS	M	NS	NS	NS	NS	NS	NS	NS
$v_{PYR \rightarrow LAC}$	NS	L	M	M	NS	NS	NS	NS	NS	NS
$v_{LAC \rightarrow PYR}$	NS	NS	L	L	NS	NS	NS	NS	NS	NS
$v_{EAC \rightarrow AC_{CoA}}$	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
$v_{PYR \rightarrow AC_{CoA}}$	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
$v_{CIT \rightarrow \alpha-KG}$	NS	NS	NS	NS	NS	NS	M	NS	NS	NS
$v_{\alpha-KG \rightarrow SCA}$	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
$v_{SUC \rightarrow MAL}$	NS	L	NS	NS	NS	NS	M	NS	NS	NS
$v_{MAL \rightarrow OAA}$	NS	NS	L	NS	NS	NS	M	NS	NS	NS
$v_{O_2 \rightarrow H_2O}$	NS	NS	L	NS	NS	NS	L	NS	NS	L

Table 5

Overall sensitivity to the C_c parameters: H: high, M: moderate, L: low, NS: not sensitive.

C_c	GLY	LAC	NAD ⁺ _c	NADH _c	ADP _c	ATP _c	NAD ⁺ _m	NADH _m	ADP _m	ATP _m
$C_{c, GLU}$	NS	L	L	L	NS	NS	NS	NS	NS	NS
$C_{c, G6P}$	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
$C_{c, GLY}$	M	NS	L	M	NS	NS	NS	NS	NS	NS
$C_{c, GAP}$	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
$C_{c, BPg}$	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
$C_{c, PYR}$	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

$C_{c,LAC}$	NS	M	NS	NS	NS	NS	NS	NS	NS	NS	NS
$C_{c,TG}$	NS	NS	L	NS	NS	NS	NS	NS	NS	NS	NS
$C_{c,GLP}$	NS	NS	L	NS	NS	NS	NS	NS	NS	NS	NS
$C_{c,FEA}$	NS	NS	L	NS	NS	NS	NS	NS	NS	NS	NS
$C_{c,EAC}$	NS	NS	L	NS	NS	NS	NS	NS	NS	NS	NS
$C_{c,CoA}$	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
$C_{c,PzP}$	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
$C_{c,Cr}$	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
$C_{c,NAD}$	NS	NS	M	M	NS	NS	NS	NS	NS	NS	NS
$C_{c,NADH}$	NS	NS	NS	M	NS	NS	NS	NS	NS	NS	NS
$C_{c,ATP}$	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
$C_{c,ADP}$	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
C_{c,P_i}	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
$C_{c,AAA}$	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
$C_{c,MAL}$	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Table 6

Overall sensitivity to the C_m parameters: H: high, M: moderate, L: low, NS: not sensitive.

C_m	GLY	LAC	NAD^+_c	$NADH_c$	ADP_c	ATP_c	NAD^+_m	$NADH_m$	ADP_m	ATP_m
$C_{m,PYP}$	NS	NS	M	NS	NS	NS	NS	NS	NS	NS
$C_{m,EAC}$	NS	NS	L	M	NS	NS	NS	NS	NS	NS
$C_{m,ACoA}$	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
$C_{m,bADP}$	H	M	M	M	H	L	NS	NS	NS	NS
$C_{m,Cr}$	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
$C_{m,CIT}$	NS	NS	L	L	NS	NS	M	M	NS	NS
$C_{m,\alpha-KG}$	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
$C_{m,SCA}$	NS	NS	L	NS	NS	NS	NS	NS	NS	NS
$C_{m,SUC}$	NS	NS	M	L	NS	NS	M	NS	NS	NS
$C_{m,MAL}$	NS	NS	M	L	NS	NS	M	NS	NS	NS
$C_{m,AAA}$	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
$C_{m,CoA}$	NS	NS	M	L	NS	NS	L	L	NS	NS
C_{m,O_2}	NS	L	L	L	L	NS	NS	NS	NS	NS
C_{m,CO_2}	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
$C_{m,NADH}$	NS	NS	NS	NS	NS	NS	L	L	NS	NS
$C_{m,NAD}$	NS	NS	NS	NS	NS	NS	M	NS	NS	NS
$C_{m,ATP}$	NS	NS	NS	NS	NS	NS	NS	NS	M	NS
$C_{m,ADP}$	NS	NS	NS	NS	NS	NS	NS	NS	M	NS
C_{m,P_i}	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Table 7

Overall sensitivity to the C_b parameters: H: high, M: moderate, L: low, NS: not sensitive.

C_b	GLY	LAC	NAD^+_c	$NADH_c$	ADP_c	ATP_c	NAD^+_m	$NADH_m$	ADP_m	ATP_m
$C_{b,GLU}$	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
$C_{b,LAC}$	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
$C_{b,FEA}$	NS	NS	M	NS	NS	NS	NS	NS	NS	NS
C_{b,O_2}	NS	M	M	M	NS	NS	L	NS	NS	NS
C_{b,CO_2}	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
$C_{b,PYP}$	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Table 8

Overall sensitivity to the T parameters: H: high, M: moderate, L: low, NS: not sensitive.

T	GLY	LAC	NAD^+_c	$NADH_c$	ADP_c	ATP_c	NAD^+_m	$NADH_m$	ADP_m	ATP_m
$T_{GLU}^{b \rightarrow c}$	L	NS	M	M	NS	NS	NS	NS	NS	NS
$T_{LAC}^{b \rightarrow c}$	NS	M	NS	NS	NS	NS	NS	NS	NS	NS
$T_{LAC}^{c \rightarrow b}$	NS	H	NS	NS	NS	NS	NS	NS	NS	NS
$T_{PYP}^{c \rightarrow m}$	NS	M	H	M	NS	NS	NS	NS	NS	NS
$T_{EAC}^{c \rightarrow m}$	NS	M	M	M	NS	NS	M	M	NS	NS

T	GLY	LAC	NAD_c^+	NADH_c	ADP_c	ATP_c	NAD_m^+	NADH_m	ADP_m	ATP_m
$T_{\text{CoA}}^{c \rightarrow m}$	NS	M	M	M	NS	NS	M	M	NS	NS
$T_{\text{P}_i}^{c \rightarrow m}$	H	M	M	L	NS	NS	NS	NS	NS	NS
$T_{\text{ATP}}^{m \rightarrow c}$	H	M	H	H	H	M	M	NS	H	H
$T_{\text{ADP}}^{c \rightarrow m}$	H	H	H	H	H	M	M	NS	H	M
$T_{\text{NADH}}^{c \rightarrow m}$	L	M	H	H	NS	NS	H	M	NS	NS
$T_{\text{NAD}}^{m \rightarrow c}$	NS	M	H	H	NS	NS	H	M	NS	NS

Table 9

Overall sensitivity to the M parameters: H: high, M: moderate, L: low, NS: not sensitive.

M	GLY	LAC	NAD_c^+	NADH_c	ADP_c	ATP_c	NAD_m^+	NADH_m	ADP_m	ATP_m
$M_{\text{GLU}}^{b \rightarrow c}$	NS	NS	L	NS	NS	NS	NS	NS	NS	NS
$M_{\text{LAC}}^{b \rightarrow c}$	NS	M	NS	L	NS	NS	NS	NS	NS	NS
$M_{\text{LAC}}^{c \rightarrow b}$	M	M	H	M	NS	NS	NS	H	NS	NS
$M_{\text{PYR}}^{c \rightarrow m}$	NS	M	M	M	NS	NS	NS	NS	NS	NS
$M_{\text{FAC}}^{c \rightarrow m}$	NS	M	M	M	NS	NS	M	M	NS	NS
$M_{\text{CoA}}^{c \rightarrow m}$	NS	M	M	M	NS	NS	M	M	NS	NS
$M_{\text{P}_i}^{c \rightarrow m}$	H	L	M	M	NS	NS	NS	NS	NS	NS
$M_{\text{ATP}}^{m \rightarrow c}$	H	M	H	M	H	M	M	NS	H	M
$M_{\text{ADP}}^{c \rightarrow m}$	H	M	H	M	H	M	M	NS	H	M
$M_{\text{NADH}}^{c \rightarrow m}$	NS	M	H	M	H	NS	M	H	NS	NS
$M_{\text{NAD}}^{m \rightarrow c}$	NS	M	H	M	H	NS	M	H	NS	NS

Table 10

Overall sensitivity to the λ parameters: H: high, M: moderate, L: low, NS: not sensitive.

λ	GLY	LAC	NAD_c^+	NADH_c	ADP_c	ATP_c	NAD_m^+	NADH_m	ADP_m	ATP_m
$\lambda_{\text{FFA}}^{b \rightarrow c}$	NS	M	H	M	M	NS	M	M	NS	NS
$\lambda_{\text{O}_2}^{b \rightarrow c}$	M	M	H	M	M	M	M	M	M	M
$\lambda_{\text{CO}_2}^{c \rightarrow b}$	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
$\lambda_{\text{PYR}}^{b \rightarrow c}$	NS	M	M	NS	NS	NS	NS	NS	NS	NS

Table 11

Overall sensitivity to the σ parameters: H: high, M: moderate, L: low, NS: not sensitive.

σ	GLY	LAC	NAD_c^+	NADH_c	ADP_c	ATP_c	NAD_m^+	NADH_m	ADP_m	ATP_m
$\sigma_{\text{FFA}}^{b \rightarrow c}$	NS	L	M	M	NS	NS	M	M	NS	NS_{CO}
$\sigma_{\text{O}_2}^{b \rightarrow c}$	NS	L	M	NS	M	M	M	M	M	M
$\sigma_{\text{CO}_2}^{c \rightarrow b}$	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
$\sigma_{\text{PYR}}^{b \rightarrow c}$	NS	NS	L	L	L	NS	NS	NS	NS	NS

Table 12
The metabolic reactions fluxes.

Cytosol Reaction	Stoichiometry
Φ_1 GLU utilization	$\text{GLU} + \text{ATP} \rightarrow \text{G6P} + \text{ADP} + \text{H}^+$
Φ_2 GLY synthesis	$\text{G6P} + \text{ATP} \rightarrow \text{GLY} + \text{ADP} + 2\text{P}_i$
Φ_3 GLY utilization	$\text{GLY} + \text{P}_i \rightarrow \text{G6P}$
Φ_4 G6P breakdown	$\text{G6P} + \text{ATP} \rightarrow 2\text{GAP} + \text{ADP}$
Φ_5 GAP breakdown	$\text{GAP} + \text{NAD}^+ + \text{P}_i \rightarrow \text{BPG} + \text{NADH} + \text{H}^+$
Φ_6 PYR formation	$\text{BPG} + 2\text{ADP} \rightarrow \text{PYR} + 2\text{ATP}$
Φ_7 PPYR reduction	$\text{PYR} + \text{NADH} + \text{H}^+ \rightarrow \text{LAC} + \text{NAD}^+$
Φ_8 LAC oxidation	$\text{LAC} + \text{NAD}^+ \rightarrow \text{PYR} + \text{NADH} + \text{H}^+$
Φ_9 TG oxidation	$\text{TG} \rightarrow 3 \text{FFA} + \text{GLR}$
Φ_{10} TG synthesis	$3 \text{FA} + \text{GLR} + 3\text{ATP} \rightarrow \text{TG} + 3\text{ADP} + 3\text{P}_i$
Φ_{11} FFA activation	$\text{FFA} + \text{CoA} + 2\text{ATP} \rightarrow \text{FAC} + 2\text{ADP} + 2\text{P}_i$
Φ_{12} PCr breakdown	$\text{PCr} + \text{ADP} + \text{H}^+ \rightarrow \text{Cr} + \text{ATP}$
Φ_{13} PCr synthesis	$\text{Cr} + \text{ATP} \rightarrow \text{PCr} + \text{ADP} + \text{H}^+$
Φ_{14} ATP Utilization	$\text{H}_2\text{O} + \text{ATP} \rightarrow \text{ADP} + \text{H}^+ + \text{P}_i$
Φ_{15} ADP Conversion	$\text{ATP} \rightarrow \text{bADP}$
Φ_{16} ADP Conversion	$\text{bADP} \rightarrow \text{ATP}$
Mitochondria Reaction	Stoichiometry
Φ_{17} β -oxidation	$\text{FAC} + 7 \text{CoA} + \frac{35}{3} \text{NAD}^+ \rightarrow 8\text{ACoA} + \frac{35}{3} \text{NADH} + 7\text{H}^+$
Φ_{18} PYR oxidation	$\text{PYR} + \text{CoA} + \text{NAD}^+ \rightarrow \text{ACoA} + \text{NADH} + \text{CO}_2 + \text{H}^+$
Φ_{19} CIT formation	$\text{ACoA} + \text{OAA} + \text{H}_2\text{O} \rightarrow \text{CIT} + \text{CoA} + \text{H}^+$
Φ_{20} α -KG formulation	$\text{CIT} + \text{NAD}^+ \rightarrow \alpha\text{-KG} + \text{NADH} + \text{CO}_2 + \text{H}^+$
Φ_{21} SCA formation	$\alpha\text{-KG} + \text{CoA} + \text{NAD}^+ \rightarrow \text{SCA} + \text{NADH} + \text{CO}_2 + \text{H}^+$
Φ_{22} SUC formation	$\text{SCA} + \text{ADP} + \text{P}_i \rightarrow \text{SUC} + \text{ATP} + \text{CoA}$
Φ_{23} MAL formation	$\text{SUC} + \frac{2}{3} \text{NAD}^+ \rightarrow \text{MAL} + \frac{2}{3} \text{NADH}$
Φ_{24} OAA formation	$\text{MAL} + \text{NAD}^+ \rightarrow \text{OAA} + \text{NADH} + \text{H}^+$
Φ_{25} Oxidative phosphorylation	$\frac{1}{2} \text{O}_2 + 3\text{ADP} + 3\text{P}_i + \text{NADH} + \text{H}^+ \rightarrow \text{H}_2\text{O} + 3\text{ATP} + \text{NAD}^+$

Table 13
The metabolic reaction rates.

	Cytosol Reaction Rates
R_{GLU}^c	$-\Phi_1$
R_{G6P}^c	$\Phi_1 + \Phi_3 - \Phi_2 - \Phi_4$
R_{GLY}^c	$\Phi_2 - \Phi_3$
R_{GAP}^c	$2\Phi_4 - \Phi_5$
R_{BPG}^c	$\Phi_5 - \Phi_6$
R_{LAC}^c	$\Phi_7 - \Phi_8$
R_{TG}^c	$\Phi_{10} - \Phi_9$
R_{GLR}^c	$\Phi_9 - \Phi_{10}$
R_{FFA}^c	$3\Phi_9 - 3\Phi_{10} - \Phi_{11}$
R_{PCr}^c	$-\Phi_{12} + \Phi_{13}$
R_{Cr}^c	$-\Phi_{12} - \Phi_{13}$
R_{PYR}^c	$\Phi_6 + \Phi_8 - \Phi_7$
R_{FAC}^c	Φ_{11}
R_{bADP}^c	$\Phi_{15} - \Phi_{16}$
R_{ATP}^c	$-\Phi_1 - \Phi_2 - \Phi_4 + 2 - \Phi_6 - \Phi_{10} - \Phi_{11} - \Phi_{14} + \Phi_{12} - \Phi_{13}$
R_{ADP}^c	$\Phi_1 + \Phi_2 + \Phi_4 - 2 - \Phi_6 + \Phi_{10} + \Phi_{11} + \Phi_{14} - \Phi_{12} + \Phi_{13} - \Phi_{15} + \Phi_{16}$
R_{NAD}^c	$-\Phi_5 + \Phi_7 - \Phi_8$
R_{NADH}^c	$\Phi_5 - \Phi_7 + \Phi_8$
$R_{\text{P}_i}^c$	$2\Phi_2 - \Phi_3 - \Phi_5 - \Phi_{14} + \Phi_{11}$
R_{CoA}^c	$\Phi_5 - \Phi_7 + \Phi_8$

Cytosol Reaction Rates

Mitochondria Reaction Rates	
R_{ACoA}^m	$\Phi_{18} - 8\Phi_{17}$
R_{CIT}^m	$\Phi_{19} - \Phi_{20}$
$R_{a\text{-KG}}^m$	$\Phi_{20} - \Phi_{21}$
R_{SCA}^m	$\Phi_{21} - \Phi_{22}$
R_{SUC}^m	$\Phi_{22} - \Phi_{23}$
R_{PYR}^m	$-\Phi_{18}$
R_{FAC}^m	$-\Phi_{17}$
R_{MAL}^m	$\Phi_{23} - \Phi_{24}$
R_{OAA}^m	$\Phi_{24} - \Phi_{19}$
R_{ATP}^m	$\Phi_{22} - 6\Phi_{25}$
R_{ADP}^m	$-\Phi_{22} + 6\Phi_{25}$
R_{NAD}^m	$2\Phi_{25} - \frac{35}{3}\Phi_{17} - \frac{2}{3}\Phi_{23} - \Phi_{20} - \Phi_{21} - \Phi_{24} - \Phi_{18}$
R_{NADH}^m	$-2\Phi_{25} + \frac{35}{3}\Phi_{17} + \frac{2}{3}\Phi_{23} + \Phi_{20} + \Phi_{21} + \Phi_{24} + \Phi_{18}$
$R_{P_i}^m$	$-\Phi_{22} + 6\Phi_{25}$
R_{CoA}^m	$\Phi_{19} + \Phi_{22} - \Phi_{18} - 7\Phi_{17} - \Phi_{21}$
$R_{\text{O}_2}^m$	$-\Phi_{25}$
$R_{\text{CO}_2}^m$	$\Phi_{18} + \Phi_{20} + \Phi_{21}$

Table 14

The transport rates.

Facilitated	Passive
$J_{b \rightarrow c, \text{GLU}}^f$	$J_{b \rightarrow c, \text{FFA}}^P$
$J_{b \rightarrow c, \text{LAC}}^f$	$J_{b \rightarrow c, \text{O}_2}^P$
$J_{c \rightarrow b, \text{LAC}}^f$	$J_{c \rightarrow b, \text{CO}_2}^P$
$J_{c \rightarrow m, \text{PYR}}^f$	$J_{b \rightarrow c, \text{PYR}}^P$
$J_{c \rightarrow m, \text{FAC}}^f$	
$J_{c \rightarrow m, \text{CoA}}^f$	
$J_{c \rightarrow m, P_i}^f$	
$J_{m \rightarrow c, \text{ATP}}^f$	
$J_{c \rightarrow m, \text{ADP}}^f$	
$J_{c \rightarrow m, \text{NADH}}^f$	
$J_{m \rightarrow c, \text{NAD}}^f$	

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Figure 1. Sensitivity of the NADH in cytosol (top) and in mitochondria (bottom) to the $V_{\max}$ and K parameters at the conditional mean (left) and at the start of the Markov chain (right).

Figure 2. 50% and 90% confidence envelopes for the sensitivity of glycogen to $V_{ATP \rightarrow ADP}$ and NAD^+ to the concentration of bADP (right), and the corresponding histograms (bottom) calculated over 500 realizations of the posterior sample.

Figure 3.

Schematic representation of the sensitivity of the biochemical reactions and transports to the cytosolic Lactate and Glycogen, red: high, purple: moderate, green: low, gray: not sensitive.

Figure 4.

Schematic representation of the sensitivity of the enzyme concentrations NAD^+ , NADH , ATP and ADP in the cytosol and mitochondria, red: high, purple: moderate, green: low, gray: not sensitive.