Critical Reviews™ in Biomedical Engineering, 39(5):363–377 (2011)

Computational Modeling of Mitochondrial Energy Transduction

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ABSTRACT: Mitochondria are the power plant of the heart, burning fat and sugars to supply the muscle with the adenosine triphosphate (ATP) free energy that drives contraction and relaxation during each heart beat. This function was first captured in a mathematical model in 1967. Today, interest in such a model has been rekindled by ongoing *in silico* integrative physiology efforts such as the Cardiac Physiome project. Here, the status of the field of computational modeling of mitochondrial ATP synthetic function is reviewed.

KEY WORDS: computational modeling, mitochondria, metabolism, mammalian

I. INTRODUCTION

The cyclic contraction and relaxation of the beating heart that pumps blood through the cardiovascular system of the human body is powered by the energy of adenosine triphosphate (ATP) hydrolysis. This thermodynamic potential, defined as $\Delta Gp = \Delta Gp^{o'} + RT \ln([ATP]/[ADP]*[Pi])$, is maintained more or less constant at -58 kJ/mol in living heart muscle.[1] This energy is largely the excess of the equilibrium potentials of the molecular machines that power contraction and relaxation of the heart at the cellular level (myosin adenosine triphosphatase [ATPase] and sarcoplasmic reticular calcium ATPase, respectively; -45 and -48 kJ/mol, respectively).[2,3] Mitochondria are the main source of ATP in cardiac muscle that maintains ΔGp at its proper value during work.[1] The massive amounts of the hydrolysis products adenosine diphosphate (ADP) and inorganic phosphate (Pi) that are produced during each heartbeat in the cardiomyocyte are taken up from the cytoplasm by the mitochondria and chemically coupled to form ATP in a metabolic process termed *oxidative phosphorylation*.[4] Here, the energy stored in chemical bonds in fatty acids, ketone bodies, and monocarboxylic acids derived from glycogen breakdown is transduced to ATP by a network of proteins, consuming oxygen and producing carbon dioxide in the process.[4]Much is known about the molecular details of mitochondrial energy transduction (MET).[4] The inner membrane of the double membrane that envelops the mitochondrial matrix plays a central role in MET, providing a scaffold for the core molecular machinery as well as a physical barrier for protons that is instrumental in the chemiosmotic coupling of 2 branches in the MET pathway. Specifically, the chemical energy in the pyridine and flavine nucleotides (NADH and $FADH_2$, respectively) produced by the Krebs cycle in the mitochondrial matrix is first transduced to a proton gradient across the inner membrane by a network of protein complexes known as the electron transfer chain (ETC). The energy in this proton motive force is next transduced to organic phosphate bonds in ATP molecules by the protein complex F_0F_1-ATPase and is exported to the cellular milieu by the adenine nucleotide translocator (ANT).[4]A complete account of the MET pathway should include all of the biochemical and electrochemical reactions outlined above as well as any interaction with other metabolic, transport, or signaling networks in the cell that may modulate its function. For example, the MET pathway is coupled to glycolysis[4] and the urea cycle.[4] Likewise, the mitochondrial calcium buffering function[5]interacts

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with MET via its effect on the electrochemical potential across the inner membrane ($\Delta\Psi$).[6] Many of the proteins in the ETC recently have been shown to be subject to posttranslational modification including serine phosphorylation[7] and acetylation.[8] Furthermore, the degree of protein phosphorylation was shown to change in disease states.[7] Finally, it has been well documented that the outer mitochondrial membrane is anything but permeable to many organic and inorganic ions, including ATP,[9] and may in fact play a significant regulatory role.[9,10]Capturing this accumulated knowledge of MET in a computational model, therefore, presents a daunting challenge. Yet demand for such models is on the rise in the present era of Systems Biology that seeks to deepen our understanding of living organisms by employing computational modeling to integrate the wealth of information in biology.[11] A computational MET model will be a crucial building block in any eukaryotic *in silico* cell or organ effort such as that undertaken, for example, by the IUPS Physiome Consortium.[12] Here, we will review the literature on MET computational modeling and assess the current status of the field. The aim of this review is, however, not to identify "the best" MET computational model that is currently available. As in any modeling problem, the merit of every model that has been developed can and should be judged only within the context of the particular biological question being investigated, for, in their very essence, computational models are quantitative formulations of hypotheses.[13] Therefore, we will instead review what questions about MET have been investigated using computational modeling as an investigative tool and what progress has been made with respect to both understanding the biology of MET as well as the science, and art, of modeling itself.

II. HISTORICAL OVERVIEW: QUESTIONS AND MODELS OF MITOCHONDRIAL ENERGY TRANSDUCTION

Figure 1 shows the block diagram representation of the very first mathematical model of MET, developed by E. M. Chance[14] in 1967 that was formulated in terms of "operational flux expressions" devoid of any mechanistic detail of underlying enzyme-catalyzed reactions to test a preliminary hypothesis

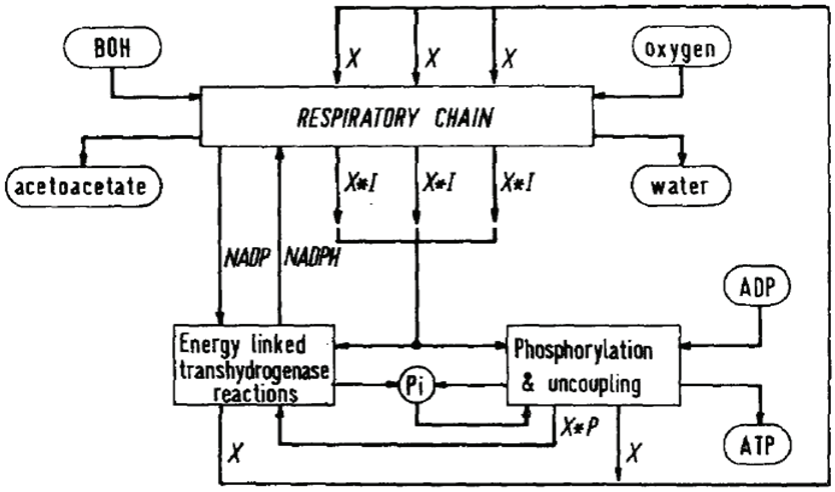

FIGURE 1. Block diagram of the metabolic processes included in the computational model of MET. The rectangular blocks represent groups of reactions describing the chemistry of the process. Oblong blocks represent substrate stores. Intermediates that are common to 2 or more metabolic processes are indicated by the relevant arrows, which indicate the direction of metabolic flow. The symbol * in this diagram refers to squiggle (~). Reproduced from Ref. 14 with permission from Elsevier Limited.

for the control of oxidative ADP phosphorylation. This pioneering effort was driven in part by the technological breakthrough in those days of machine computing.[15] Spurred by the next revolution in computing 10 years later (i.e., the desktop computer), in the early 1980s German scholars developed the first detailed kinetic MET models[16–18] and laid the foundation for many of the MET models in use today (see, e.g., Refs. 6 and 19–22; Fig. 2). The increasing sophistication (and thereby complexity) of computational MET models since these early days, as measured by the number of model parameters, is shown in Table 1. Table 2 shows the top 5 category list of biological questions that have been addressed by MET models.

The great majority of these models have sought to answer the very same question that prompted Chance's pioneering effort: What controls the rate

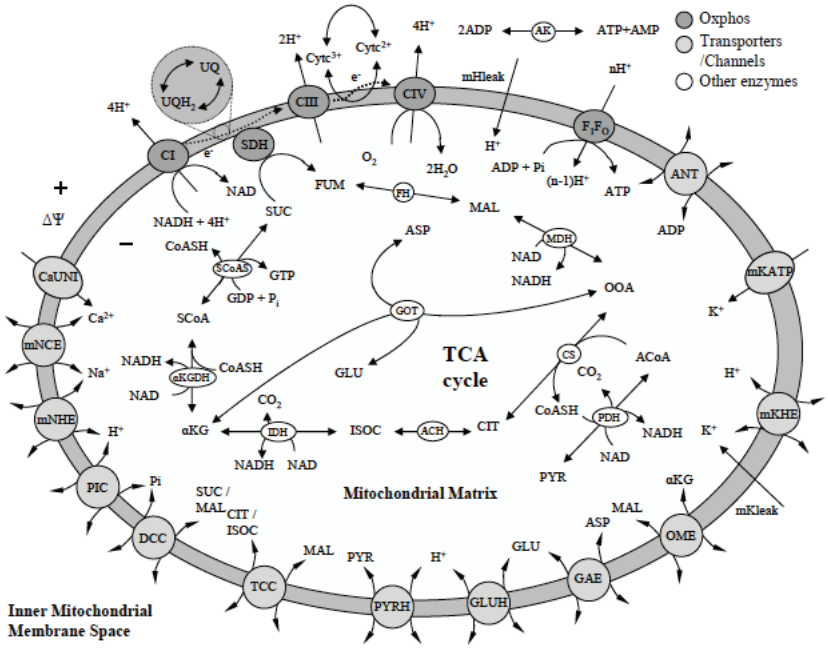

FIGURE 2. Graphic representation of the bioenergetic elements and processes described by the model. Abbreviations: Oxphos, oxidative phosphorylation elements; PYR, pyruvate; CoASH, coenzyme A; AcCoA, acetyl-coenzyme A; CIT, citrate; ISOC, isocitrate; αKG, α-ketoglutarate; SCoA, succinyl CoA; SUC, succinate; FUM, fumarate; MAL, malate; OAA, oxaloacetate; GLU, glutamate; ASP, aspartate; NADH, reduced nicotinamide adenine nucleotide; NAD, oxidized nicotinamide adenine nucleotide; GTP, guanidine triphosphate; GDP, guanidine diphosphate; Pi, inorganic phosphate; UQ, ubiquinone; UQH_2, ubiquinol; $Cytc^{3+}$, oxidized cytochrome c; $Cytc^{2+}$, reduced cytochrome c; PDH, pyruvate dehydrogenase; CS, citrate synthase; ACH, aconitase; IDH, isocitrate dehydrogenase; αKGDH, α-ketoglutarate dehydrogenase; ScoAS, succinyl CoA synthetase; SDH, succinate dehydrogenase; FH, fumarate hydratase; MDH, malate dehydrogenase; GOT, glutamate oxaloacetate transaminase; CI, complex I; CIII, complex III; CIV, complex IV; mHleak, proton leak; F_1F_o, F_1F_o ATPase; ANT, adenine nucleotide transporter; PIC, inorganic phosphate carrier; GAE, glutamate/aspartate exchanger; OME, α-ketoglutarate/malate exchanger; DCC, dicarboxylate carrier; TCC, tricarboxylate carrier; PYRH, pyruvate-proton cotransporter; GLUH, glutamate-proton cotransporter; mKATP, ATP-dependent K^+ channel; mKHE, K^+/H^+ exchanger; mKleak, K^+ leak; mNHE, Na^+/H^+ exchanger; mNCE, Na^+/Ca^{2+} exchanger; CaUNI, Ca^{2+} uniporter; AK, adenylate kinase. Reproduced from Ref. 19 with permission from the authors.

TABLE 1. Overview of the Number of State Variables and Parameters, Including Metabolite Poolsizes, in Various Computational Models of Mitochondrial Energy Transduction

Model	No. of State Variables	No. of Parameters	Reference
Chance 1967	9	17	14
Bohnensack 1981	8	20[a]	16
Holzhutter 1985	9	27	17
Magnus & Keizer 1997	3	52	6
Cortassa 2003	12	99	21
Wu 2007	64	210	22
Bazil 2010	73	359	19

[a] The article explicitly reports 14 parameters; careful inspection of the model, however, shows that 6 more quantitative assumptions/relations were used in the simulations.[16]

of mitochondrial ATP synthesis? This is not surprising, for various reasons. First of all, mitochondrial respiratory control and energy homeostasis are fundamental issues in eukaryotic cell biology. Secondly, computational modeling is particularly well-suited to investigate this type of problem in the philosophical tradition of Occam's razor; that is, start by capturing the simplest hypothesis in a mathematical formulation and then test its predictions against experimental observation. Indeed, between 1985 and 1995, detailed kinetic MET modeling was all but abandoned in favor of highly simplified, lumped (i.e., single equation) MET models to test various respiratory control hypotheses[2,23–30] (but see also Refs. 31 and 32). Specifically, feedback (i.e., mediated by secondary reporters of ATP turnover changes such as the cellular concentrations of ADP and Pi[27–32]) versus feed-forward (i.e., mediated by primary reporters such as the calcium concentration in excitable tissues[27–29]) respiratory control mechanisms were investigated heavily. Notably, this particular episode and direction of MET modeling was led by biochemists and physiologists rather than mathematicians and biologists, driven either by a desire to understand revolutionary in vivo observations of ATP metabolism in skeletal and cardiac muscle by ^{31}P nuclear magnetic resonance spectroscopy[23,26–30] or by the surge of interest at the time in Metabolic Control theory.[2,25]

The present era of Computational and Systems Biology has witnessed a return to detailed computational MET modeling, led in part by mathematicians and bioengineers (see, e.g., Refs. 19–22 and 33). This has resulted in an explosion of complexity of the models (Table 1). First, Magnus and Keizer[6] introduced calcium and sodium transport in MET modeling to study its interaction with energetics. Cortassa and coworkers[21] next introduced a detailed kinetic model of the Krebs cycle to test quantitatively the contribution of feed-forward control of mitochondrial respiration to energy balance in cardiomyocytes mediated by calcium stimulation of substrate oxidation.* The relevance of their conclusions was, however, hampered by the poor homeostatic performance of the model with respect to the intramitochondrial redox potential during active respiration.[21] Beard[20] instead opted to incorporate new insights on Pi stimulation of MET and omit any calcium transport to test if feedback control of respiration suffices to explain energy balance in cardiomyocytes. A more fundamental advance in MET modeling introduced by Beard[20] has been in the model design itself: by its rigorous rooting

*Korzeniewski also explored this respiratory control mechanism in his numerical studies of energy balance in striated muscle (e.g. [31;32]) but using phenomenological rather than mechanistic modeling.

TABLE 2. Top 5 List of Biological Questions Addressed By Computational Models of Mitochondrial Energy Transduction

Biological Question	First study	Reference	Total Articles
Mitochondrial ATP flux control	1967	14	>20
Mitochondrial calcium handling	1997	6	9
Mitochondrial ATP flux capacity	1977	71	4
Mitochondrial ROS production	2009	33	2
Mitochondrial volume dynamics	2010	19	1

Abbreviations: ATP, adenosine triphosphate; ROS, reactive oxygen species.

in biophysical principles to gain thermodynamic constraints on the solution space, the physiological behavior of the MET model was improved greatly.[19,22] An important outcome of this work was the finding that the predicted stationary states of energy balance in cardiac and skeletal muscle were in good agreement with experimental data.[22,34] This result was taken as strong evidence that feedback control of respiration is the principle metabolic control mechanism in MET.[35]

Although the investigation of metabolic control in MET undoubtedly has benefitted from the explicit, quantitative formulation and test of leading hypotheses that is at the very heart of computational modeling,[13] several trends in recent MET modeling warrant some caution. First and foremost, the rate at which the molecular detail and complexity of MET models has recently been expanding (Table 1) has far exceeded the rate at which the experimental database supporting MET model parameterization and validation has expanded. For example, the most recent adaptation and expansion of the 2007-generation MET model from the Beard group[22] by Bazil and colleagues[19] required an increase of the number of model parameters from 210 to 359 (Table 1). Of the 210 parameters in the original model alone, a total of 151 (60 kinetic parameters and 91 maximal rate terms) were adjusted on the basis of component model fitting to 83 data curves retrieved from 9 new independent datasets[19] (see specifically the supplemental material S3 of Ref 19). Secondly, many MET models have been tested exclusively against sparse datasets of stationary metabolic states.[19–22,34] That this is indeed a concern was demonstrated in a recent test of the 2006 generation of the Beard MET model against rich experimental datasets on ATP metabolism dynamics in skeletal muscle.[36] The test revealed a significant shortcoming in the model with respect to mitochondrial ADP sensing.[36] The problem was fixed partly by reparameterization of the model directed by advanced model analysis[36] (see section V, Model Analysis). Third, the majority of current MET models are highly deterministic despite many sources of error (e.g., in vitro experimental models, problems with identifying parameters) in the typically macroscopic kinetic measurements that have been used to parameterize the models.[37] The impact of any parameter uncertainties on the reliability of the model predictions and any conclusions that are drawn on its basis are only rarely discussed and even more rarely quantified (see, e.g., Ref. 38). In sections III to V of this review, these problematic trends will be discussed in more detail.

Finally, 2 of the other categories of biological questions that have driven computational MET modeling thus far (Table 2) warrant particular mentioning. Specifically, in both cases the question arises of whether or not these modeling efforts are premature in light of the concerns about contemporary MET modeling, raised above. The first category entails the subject of reactive oxygen species (ROS) production in MET.[33–39] Recently, Selivanov and colleagues[33] developed a computational model of the ETC to investigate the well-documented, paradoxical elevated ROS production during tissue reperfusion. One strikingly complex feature of the model is that it generates hundreds of differential

equations to model the redox state of complex III of the mitochondrial ETC.[33] Although the simulations identify an intriguing mechanistic explanation of the physiology, the study includes only qualitative validation of key model predictions.[33] In another subject category is the MET model proposed by Bazil and colleagues[19] that was discussed briefly already (Figure 2). The authors report that their particular modeling effort represents the "next step toward a complete physiologically faithful MET model" and was used to predict, amongst others, mitochondrial volume dynamics during metabolic activity.[19] From a philosophical point of view that Systems Biology seeks to build the framework to study the elusive genotype-phenotype relation,[11] there may, in time, indeed be a need for the type of "comprehensive" MET model to which the authors refer. Though the effort in and by itself was certainly laudable, the plethora of fundamental as well as practical problems in current MET modeling that warrant addressing raise some doubts whether that time has come.

III. COMPUTATIONAL MODEL DEVELOPMENT I: FORMALISMS AND CONCEPTUAL APPROACHES

The previous section reviewed the variety of questions in MET that have been studied using computational modeling. Close inspection of these models shows there is also a wide variety in the way these models have been formulated, ranging from simple, single-equation, lumped models to relatively complex models with >50 state variables and >200 parameters (Table 1). The principle reason for these differences is that the purpose and application are essential for the selection of the best mathematical framework.[40] In this section we will discuss some fundamental aspects of different model building strategies.

Approaches in computational modeling can be thought of as falling into 2 philosophical categories[12,13,41]: inductive versus hypothesis-driven science. The former category comprises large-scale modeling approaches for which it is assumed that important modeling features emerge from their simulations and analysis. The latter category aims for the simplest model, capturing the key features of a system consistent with the level of available experimental data. These 2 paradigms also can be recognized from the list of MET models presented in the previous section. The hypothesis-driven approach is easily recognizable among several studies investigating the control of mitochondrial respiration using single, lumped equation models,[24–26,29,30,42,43] whereas the majority of recent work follows an inductive approach, aiming for the construction of relatively large-scale mechanistic models.[6,16,19,21,22,32,33,39,44,45†]

III.A. Hypothesis-Driven MET Modeling

The means by which mitochondrial respiration has been thought to be controlled has undergone several revisions over the last decades (see a review in Ref. 49). Single, lumped equation models have been developed to test several of these hypotheses. In these studies the mitochondria were reduced to a single unit characterized by its input–output behavior. This input–output classification refers to a classic control scheme in which the inputs reflect the primary regulators and the output is mitochondrial respiration or ATP production. The mathematical equation captured a certain hypothesis (primary regulator[s] and their mechanisms of action) and, by comparing model simulations against available experimental data, it was possible to test the hypothesis. The approach is most successful (1) when testing a relatively simple, well-defined hypothesis taking into account only primary regulators and (2) if high-quality quantitative data sampling the input–output relation is available.

This strategy has been applied with some success to investigate MET regulation in muscle. It has resulted in several models describing mitochondrial input–output behavior based upon some kinetic,[24,29] hybrid kinetic/thermodynamic,[26] or purely thermo-

† In this section we will not discuss the stoichiometric modeling approach because this technique is not commonly applied to this specific problem (but see Ref. 46). For extensive reviews about stoichiometric modeling the reader is referred to Refs. 47 and 48.

dynamic mechanism.[30] The need for high-quality data was illustrated, for example, by a study by Jeneson and colleagues,[26] who concluded that the quasi linear-sigmoidal description between phosphate potential and oxidative phosphorylation flux predicted by their hybrid kinetic/thermodynamic model was statistically equivalent to a strictly linear relation. Consequently, true testing of this model/hypothesis in vivo was not possible until data with a higher sampling density became available.[36]

III.B. Inductive MET Modeling

One of the requirements of a hypothesis-driven approach is that translation of the hypothesis into a mathematical representation is relatively straightforward. However, if the hypothesis is already complicated or incomplete at the qualitative, conceptual level, translation itself becomes a challenge. In such a case an inductive research strategy can provide a suitable alternative. By reconstructing the system from submodels of its individual components, it is not necessary to have a detailed hypothesis beforehand; the hypothesis is generated while building/developing the model (see, e.g., Ref. 19). This research strategy often is applied in a mechanistic modeling approach. However, the scope of the complexity problem in this approach is daunting. For example, with respect to mitochondrial biology, recent estimates put the number of proteins that make up the organelle hardware at 3000.[50–52] Many of these proteins and their function remain to be characterized, including any interaction with MET function.

Mechanistic models seek to reconstruct networks *in silico* based upon quantitative kinetic and/or thermodynamic information of individual network components. Building computational models strictly based upon mechanistic details requires quantitative information about reaction rates and molecular concentrations. For most processes this information is not available and, consequently, a more practical approach to mechanism-based modeling often is required; biochemical data and model parameters often are collected from different species, experimental settings, and cell types.[53] Moreover, in case mechanistic information is unclear, it is necessary to fill in the gaps with preferably simple mechanisms without having any kinetic parameters available.[54] Sometimes it is possible to clamp model variables at physiological values reducing the complexity of the model. For example, in MET modeling, mathematical descriptions of Pi-transporters in the mitochondrial membranes and proton buffering by other proteins and metabolites are required, amongst others, to make matrix Pi concentration and pH state variables. To omit this degree of complexity, these variables were clamped in some MET models.[6,21,45,55] By applying this type of practical solution, multiple semimechanistic MET models have been constructed.[6,16,19,21,22,32,33,39,44,45] Analyses of these models already have yielded valuable new insights (see the previous section).

A characteristic of the mechanistic modeling approach is that the hypothesis is generated while building the model. This is, for example, clear from the work of Bazil et al.,[19] who extended an already available MET model with the primary purpose of advancing the model in addition to investigating a specific question. Semimechanistic models reflect well the current state of knowledge of the biology; also at a conceptual level, the majority of the leading hypotheses contain gaps, unknowns, and uncertainties, similar to the computation models. The primary difference with a computational model is that in a mathematical formulation the uncertainty can be explicit. This characteristic is not a weakness, per se; it can be exploited in the process of improving the hypothesis. Application of mathematical techniques (e.g., sensitivity analysis) allows researchers to identify if model predictions are highly dependent on ill-defined, nonmechanistic parts or parameters of the model.[40] In such a case, reliable and accurate quantification of these mechanisms/parameters is essential to improve the hypothesis.[40] The field is currently at a stage in which it can enter this iterative cycle of hypothesis refinement/improvement. A fine example of the application of this strategy is provided by the Beard laboratory, which has started to replace phenomenologic descriptions of key parts of their MET

model by more mechanistic ones.[20,22] For example, early generations of their MET model contained a phenomenologic lumped tricarboxylic acid (TCA) cycle, which later was replaced by a mechanistic model considering all TCA cycle intermediates.[22] Similarly, other phenomenological parts of the model, for example, ANT and complex I, have been substituted more recently by more mechanistic descriptions.[56,57] As the complexity of the model and underlying hypotheses expand, however, model analysis will become less intuitive. Proper application of sophisticated mathematical techniques will, therefore, be crucial to the success of iterative hypothesis refinement and improvement. Several of these techniques will be highlighted in section V of this review.

IV. COMPUTATIONAL MODEL DEVELOPMENT II: PARAMETERIZATION AND MODEL TESTING

IV.A. Model Parameterization

A major challenge in developing mechanistic models is model parameterization. The largest mechanistic mitochondrial models that have been developed contain up to a few hundred parameters (Table 1). The majority of the parameter values originate from 3 different sources: (1) experimentally determined values; (2) values estimated based upon some experimental data by applying a parameter estimation algorithm, and (3) values estimated in previous computational studies. Although obtaining parameter values from these sources has become common practice in the field of computational biology, this does not imply that all values found are reliable.

A first problem encountered is that parameters values have typically been determined in vitro. However, in vivo interactions with other agents not included in the in vitro assay environment (e.g., the cytoskeletal matrix) may affect enzyme behavior and influence corresponding kinetic parameters. An example of the possible impact of the difference between in vitro and in vivo conditions is provided by the work of Teusink and colleagues,[58] who reconstructed the yeast glycolysis pathway purely based upon in vitro kinetic data, but failed to reproduce in vivo observed behavior with this model. Schmitz and colleagues[38] recently obtained similar results with respect to glycolysis in muscle.

A second problem is that point values typically are not available for all model parameters, either in the literature or because of practical problems encountered when trying to obtain the values experimentally. Consequently, inferring the unknown values from (dynamic) systems behavior by applying a parameter optimization algorithm is required. The number of parameter values that can be inferred accurately from the data depends on the amount of information accessible from the data (termed *practical identifiability*) and the formulation of the model equations (termed *structural identifiability*).[37,59] When a parameter is nonidentifiable, many of the applied parameter estimation algorithms are still able to assign a value to such a parameter.[60] However, these values are often poorly constrained and nonunique and therefore provide a potentially unreliable point estimate of the parameter value.[61–63] Current mitochondrial models contain many of these nonidentifiable parameters, some of which are deeply embedded in the code. For example, Bazil et al.[19] reported a low sensitivity of the 42 adjustable parameters to the experimental data used for parameter estimation, suggesting that these parameters are poorly constrained. Unfortunately, the uncertainty in parameter estimation was not reported explicitly. Without redoing the elaborate parameter optimization procedure, it is impossible to take into account in future studies.

IV.B. Model Testing

A frequently used synonym for model testing is model validation. Karl Popper[64] stated in his famous book *The Logic of Scientific Discovery* that in the empirical science true validation of a hypothesis is not possible; hypotheses can only be *in*validated. This view is also relevant for computational models, which also are hypotheses, albeit in a mathematical formulation.[13] If model behavior is consistent with an observation it only can be concluded that the

mechanisms captured by the model are sufficient to explain the observation. However, it can never be ruled out that another mechanism not included in the model is present in vivo. Though true validation is, therefore, not possible, model testing against independent data is important to gain confidence in a model and its predictions. On the other hand, failure of a model is less accepted and therefore less commonly reported, yet may be equally informative. Such failure may indicate a need for an alternative mechanisms and/or more complexity. It provides opportunities to identify novel functions of such added biological complexity and give rise to a new iterative cycle of hypothesis refinement. Identification of the limitations of a model is, therefore, an important step in advancing available models and should be included in reports whenever possible.

V. MODEL ANALYSIS

As described and illustrated in the previous section, (computational) analysis of the proposed mathematical MET models is an essential step in model development and application. Here, 2 topics of model analysis and their application in MET modeling are discussed: (2) parameter sensitivity analysis (PSA) and (3) model prediction uncertainty.

V.A. Parameter Sensitivity Analysis

The mathematical framework most commonly applied to model mitochondria consists of a set of coupled first-order ordinary differential equation (ODE), resulting in a continuous, deterministic description of the dynamics. For each molecular species in the network (e.g., mitochondrial matrix ADP, Pi, H^+, and calcium) an ODE is introduced, derived from its mass balance. Usually only a subset of these state variables can be experimentally observed (the model output[s **y**) and possibly the output of interest is an algebraic function of some of the state variables (e.g., mitochondrial ATP synthesis rate). In both local and global PSAs (LPSA and GPSA, respectively) the effect of variation in parameter values **q** on model behavior is investigated. The response of interest of the system output(s) needs to be translated into a scalar value M(**y, q**), which subsequently can be analyzed and interpreted. Typical response characteristics that have been used include the area under the curve of the output, the amplitude and period of oscillation, the deviation from the steady-state values, and the output value after a specific amount of time.[40] A criterion of particular relevance is the sum of squared differences between the output y^0 for the reference parameter values ^{0} and the perturbed system output. Similarly the difference between model outcome and experimental data can be used. In this case there is a direct relation between PSA and parameter estimation (see section IV).

Figure 3 depicts an example of a manifold that could emerge when, for varying parameters, function M is calculated. The black dot represents a certain realization of the model, that is, a certain choice for the reference model parameter set ^{0}. Mathematically, the gradient of M describes the local steepness of the function for infinitesimal change in one of the parameters and provides the basis for LPSA methods. Based on this gradient a sensitivity coefficient can be introduced according to equation 1:

$$S_{\mathrm{q}}^{M}(i) = \frac{\mathrm{d}M_i(y)/M(y^0)}{\mathrm{d}\mathrm{q}(i)/\mathrm{q}^0(i)} \quad i = 1,\ldots,p \quad (1)$$

where $\theta(i)$ represents the parameter that is varied and δM is the change in M due to the change $\delta\theta$ in θ. This relative sensitivity coefficient is similar to the control coefficients of metabolic control analysis.[2,40] This concept can be extended by allowing the infinitesimal change to become finite and of different size. Different individual parameters subsequently can be "scanned" in a stepwise manner. This univariate analysis is shown as the red line on the manifold and as the dots in the bottom plane of Figure 3.

LPSA. LPSA has been applied routinely in MET modeling studies (see, e.g., Refs. 19, 21, and 34). For example, van Stiphout and colleagues[55] analyzed a mitochondrial model featuring, amongst others, a TCA cycle model incorporating calcium activation developed by Jafri[45] and mitochondrial

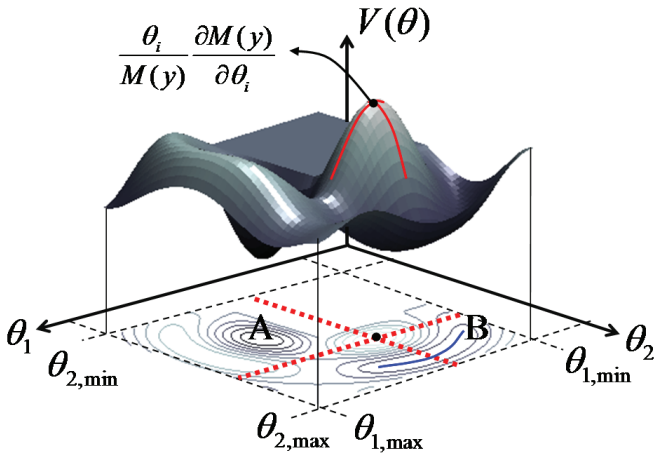

FIGURE 3. Graphic representation of 2 important aspects of model analysis: parameter sensitivity analysis and identifiability. The surface represents a manifold in parameter space for the parameters $_1$ and $_2$. For varying parameters either the sensitivity function $S_q^M(i)$ (see *equation 1*) or the cost function V(q) maps the model output y($_1$, $_2$) on a scalar value. A contour plot of isolines (lines of equal value) of the manifold is projected on the bottom plane, in which the gray represents the height of the surface (black is the lowest value, white is the highest value). A and B indicate different minima, of which A is the lowest. The parameter space is explored between bounds (min and max) for the parameters. The black dot represents a certain choice for the model parameters and the dotted lines represent a univariate analysis. See the main text for further explanation.

calcium handling and respiration developed by Magnus and Keizer.[6] van Stiphout et al.[55] identified that the model behavior (the extent to which cytosolic calcium oscillations result in oscillations in ATP and ADP through a calcium sensitive mitochondrion) was highly dependent upon only a small number of model parameters. Among these was a mitochondrial kinetic parameter *g* introduced by Magnus and Keizer[6] as a phenomenological fitting parameter to which they assigned a point value optimized for their particular biological question. Therefore, the van Stiphout[55] study teaches an important generic lesson: integration of component models in larger model frameworks (e.g., a MET model in a cardiomyocyte electrophysiology model) can only be done after careful and thorough examination of the biological question that each model addresses, including any tailored parameterization.

V.B. Model Prediction Uncertainty

Local PSA pertains to a particular point in the parameter space and one parameter is varied in each simulation. Therefore, LPSA identifies a causal relation between parameter changes and model outcome, assuming an accurate model. However, values of model parameters (e.g., rate constants) and initial conditions of the ODEs (e.g., concentrations of diverse molecules) have been derived, at best, from experimental data, but parameters also can occur for which assumed values are used, and therefore they all have a limited accuracy. To find out which of these parameter uncertainties are most critical to the models predictions, it is necessary to explore, in a probabilistic context, possibilities of nonlinear effects from simultaneous parameter variations of arbitrary magnitudes. Global PSA offers a means to do this.

Global PSA methods try to explore a large part of the parameter space through simultaneously varying all parameters, typically between a lower and upper bound. By applying a Monte Carlo simulation strategy[65] sampling parameter values randomly from the distributions, it is possible to predict a model solution space reflecting the influence of parameter uncertainty.[38] From Fig. 3 it is

clear that multivariate analyses can provide information that remains hidden in LPSA. For example, in this particular case, GPSA reveals that below certain values for $_1$ and $_2$ the model simulation does not change at all for changes in the parameters (the flat stretch of the manifold). The first application of GPSA in MET modeling was performed by Jeneson and colleagues[36] to identify which parameters in an early-generation MET model from the Beard group[34] determine the network sensitivity, denoted by parameter n_H, to a feedback control signal (changes in the extramitochondrial ADP concentration, [ADP]). The parameter n_H was estimated for each of 5000 simulations with different parameter values for the MET model and ranked according to a Kolmogorov-Smirnov test score (Fig. 4). The network sensitivity was found to be determined by only a small number of parameters and dominated by a single parameter (parameter No. 14; Fig. 4). This particular parameter, ANT θ, is a phenomenologic partition coefficient of $_m$ originally introduced by Korzeniewski and Froncisz[66] in a kinetic model of the ATP/ADP exchange reaction catalyzed by the ANT and parameterized by fitting of in vitro mitochondrial adenine nucleotide uptake data.[31] It has since been copied by many other computational MET models.[34,67] Here, GPSA identified that the particular point value for this parameter was the principal determinant of the MET model sensitivity to ADP. Any uncertainty in its value would, therefore ,have a strong impact on model performance. Conversely, this information turned out to be key in improving the quality of model prediction of dynamic states of ATP metabolism in muscle.[36]

In GPSA, statistical analyses of a large number of model simulations is performed to quantify parameter sensitivity. An alternative approach is to analyze the size of the predicted solution space for a given parameter distribution.[38] If the predicted model solution space becomes unacceptably large, more information and thus new experimental data is required. Currently, the majority of the experimental data used for MET model parameterization originates from measurements of metabolites outside the mitochondrion (e.g., ^{31}P MRS, oxygen polarography).[68] Because of technological limitations, dynamical measurements of metabolites in the inter membrane space and mitochondrial matrix are still relatively rare. The level of detail used to model intramitochondrial processes has, however, increased rapidly (Table 1). Parameterization and

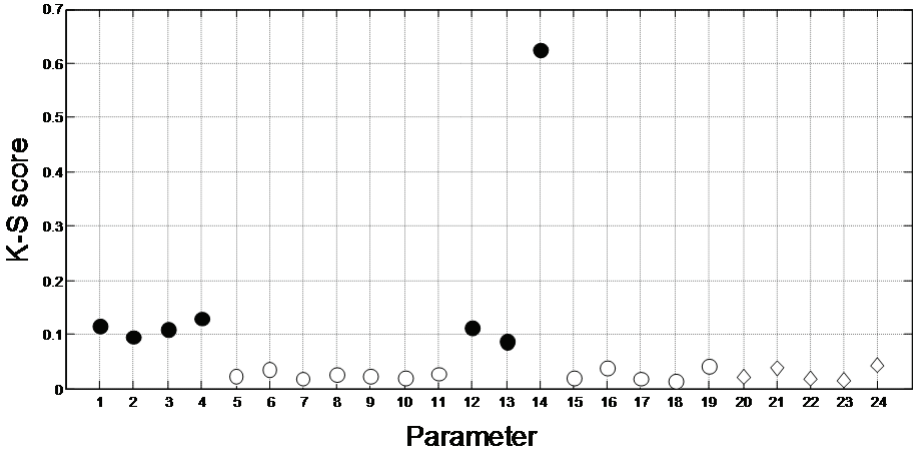

FIGURE 4. Global parameter sensitivity analysis of early generation Beard MET model. Kolmogorov-Smirnov (K-S) scores of the 19 model parameters (¡) and 5 dummy parameters (à) from the global parameter sensitivity anlysis of the model by Jeneson et al.[36] for n_H, a macroscopic parameter of the mitochondrial transduction function. Solid symbols denote parameters with a significant K-S score (threshold of significance set by the K-S scores of the 5 dummy parameters, parameters 20–24).

testing of these MET models with only limited information regarding these dynamics is very challenging. Innovative solutions providing the methodology for recording this information will be a crucial step in the progress of the field.

VI. PERSPECTIVE

The field of computational modeling of MET has witnessed a surge of new activity in recent years. Since Chance's pioneering study more than 40 years ago, much progress has been made. Current models allow for detailed simulation of many molecular processes in the pathway including acetyl-coenzyme A oxidation in the Krebs cycle, generation of proton motive force and electrochemical potential across the inner mitochondrial membrane, its interaction with anion and cation transport including calcium, and, of course, ATP production and export to the cellular milieu, as well as osmolarity and associated matrix volume changes. Application of these models to address longstanding biological questions has advanced understanding of mitochondrial respiratory control,[34–36] the interaction of mitochondrial calcium buffering function with ATP synthesis function of the organelle,[6,21,45] mitochondrial swelling during active respiration,[19] and paradoxical elevated ROS production during reperfusion.[33]

This advance has, however, come with a significant complexity price tag. The most advanced MET model to date features no less than 359 parameters (Table 1). Many of these have been given a point value on the basis of numerical curve fitting to typically noisy and sparse experimental data obtained in vitro at nonphysiological temperature from mitochondrial populations or membrane fractions isolated from various tissues (e.g., muscle, liver, heart) from different mammalian species (e.g., pigeon, rat, swine) and even yeast.[19] This raises significant concerns about the reliability of the predictions of these highly complex MET models and, as a result, any conclusions regarding mitochondrial biology and physiology based upon the simulations. Beard's innovative approach to build in thermodynamic constraints of the model solution space[20,22,69,70] constitutes one way forward in this problem. Numeric approaches such as GPSA and parameter distribution sampling (discussed in section V of this review) offer a more generic solution to the problem by allowing exploration and quantification of the impact of parameter uncertainty on model behavior and prediction. A recent first application of GPSA to MET modeling was highly promising and succeeded in rigorous identification of which parameters governed model behavior with respect to a particular aspect of metabolic regulation of ATP synthetic function.[36] Standard incorporation of these numeric model analysis techniques in any computational MET modeling should provide a solid, quantitative information base to guide (1)which model predictions are significant and therefore have biological significance, and (2) what new experimental data are required to improve the model. Without such a solid information base, any continuation of the trend of recent years—that MET model complexity doubles every generation (Table 1)—runs the paradoxical risk of yielding less instead of more biological information.

ACKNOWLEDGMENTS

The authors gratefully acknowledge generous sharing of information on their MET models by Sonia Cortassa, Fan Wu, and Jason Bazil and discussions with Dan Beard and Peter Hilbers. This work was performed in part within the framework of the Center for Translational Molecular Medicine, project PREDICCT No. 01C-104, and financed in part by the Netherlands Consortium for Systems Biology, which is part of the Netherlands Genomics Initiative/Netherlands Organisation for Scientific Research, and by the National Institutes of Health through a subcontract to grant No. HL-072011.

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