Flux-balance analysis of mitochondrial energy metabolism: consequences of systemic stoichiometric constraints

RAMPRASAD RAMAKRISHNA, JEREMY S. EDWARDS, ANDREW McCULLOCH, AND BERNHARD O. PALSSON
Department of Bioengineering, University of California San Diego, La Jolla, California 92093–0412

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Mitochondrial metabolism is a critical component in the functioning and maintenance of cellular organs. The stoichiometry of biochemical reaction networks imposes constraints on mitochondrial function. A modeling framework, flux-balance analysis (FBA), was used to characterize the optimal flux distributions for maximal ATP production in the mitochondrion. The model predicted the expected ATP yields for glucose, lactate, and palmitate. Genetic defects that affect mitochondrial functions have been implicated in several human diseases. FBA can characterize the metabolic behavior due to genetic deletions at the metabolic level, and the effect of mutations in the tricarboxylic acid (TCA) cycle on mitochondrial ATP production was simulated. The mitochondrial ATP production is severely affected by TCA-cycle mutations. In addition, the model predicts the secretion of TCA-cycle intermediates, which is observed in clinical studies of mitochondrial ATP production. The model provided a systemic perspective to characterize the effect of stoichiometric constraints and specific metabolic fluxes on mitochondrial function.

Mitochondrial metabolism results from the concerted action of several biochemical reactions that are coordinate regulated. Genotypic and physiological factors interactively contribute to adversely affect mitochondrial metabolism (23). Mutations in the genetic content can lead to changes in enzyme expression and/or activity and thus alter mitochondrial function by changing fluxes of important metabolic reactions and consequently affecting the normal physiological behavior of cells, tissues, and organs. A genetic mutation that changes a flux or fluxes consequently forces the metabolic system to a new state and affects the attainment of its physiological objective. Therefore, an understanding of the systemic constraints on metabolism can provide significant insight into physiological function. The framework of flux-balance analysis (FBA) (2, 6, 20, 22) is a powerful methodology for the analysis of metabolic systems. This approach has been successfully applied to the analysis of adipocyte metabolism (6), hybridoma metabolism (1, 14), and bacterial growth (21), in which the constraints imposed by biochemical reaction stoichiometry are systemically modeled.

The application of systemic metabolic simulation approaches can significantly aid in understanding the metabolic basis of mitochondrial-linked disease. The biochemical reactions involved in mitochondrial energy defects of protein import, defects of mitochondrial DNA (mtDNA), such as point mutations, deletions, and duplications, and defects of communication between nuclear and mitochondrial genomes (multiple deletions and mtDNA deletion) (5). The physiological manifestations of these genetic defects affect the normal functioning of substrate transport, substrate use, the tricarboxylic (TCA) cycle, the respiratory chain, and oxidation/phosphorylation coupling. mtDNA has no introns and has its own independent replication, transcription, and translation systems. Mutations in mtDNA accumulate 10–20 times faster than in comparable nuclear genes (17). The accumulation of mtDNA mutations is believed to be a key factor in aging and the progression of mitochondrial diseases (15).

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metabolism function as a coordinated network subject to stoichiometric and regulatory constraints. The objective of this study was to first develop a model for mitochondrial function, using the methods of systems theory that can predict the effect of genotype changes on certain aspects of mitochondrial function. The model was to comprise of the metabolic pathways that constitute a functioning mitochondrion, including the reactions in the mitochondrial matrix, contributing reactions in the cytosol, and key shuttles. The analysis considers the constraints imposed by the stoichiometry of these biochemical reaction networks on the achievement of specific metabolic objectives. The model will be used to characterize energy metabolism on the standard substrates of mitochondrial metabolism.

As part of the model development, the genes(s) involved in the metabolic reactions considered will be detailed. This provides a framework for studying the effect of genetic changes on metabolism. The model will be used to characterize the effect of genetic mutations on mitochondrial metabolism. These simulations will be compared with literature that describes the physiology of mitochondrial disease.

METHODS

FBA. FBA is a modeling framework developed to characterize the capabilities and properties of a metabolic network. The network comprises the metabolites and the reactions they are involved in, including their formation and degradation, transport, and cellular utilization. For every metabolite (X), a material balance is derived as follows

\[
\frac{dX_i}{dt} = \sum_j s_{ij} v_j - b_i
\]

where \(s_{ij}\) is the stoichiometric coefficient associated with each flux, \(v_j\) and \(b_i\), the net transport flux of \(X_i\). This material balance under steady-state conditions will reduce to the algebraic relation

\[
\sum_j s_{ij} v_j - b_i = 0
\]

or, for all intermediates simultaneously at steady state, the individual balance equations can be rewritten in matrix form

\[
S \cdot v - b = 0
\]

where \(S\) is the stoichiometric matrix (\(m\) rows and \(n\) columns), \(v\) is the vector of \(n\) metabolic fluxes, and \(b\) is the vector representing \(m\) transport fluxes (i.e., known consumption rates, by-product production rates, and uptake rates). The matrix \(S\) is not square (\(n > m\)), and there exists a plurality of solutions for \(equation\ 1\). That is, there can be a number of feasible flux distributions satisfying these stoichiometric constraints, each representing a particular metabolic state.

Therefore, the null space, or the set of all feasible flux distributions, represents the capabilities of the metabolic genotype. The transport fluxes represent environmental conditions that, along with the genotype, define the metabolic state. However, obtaining all possible metabolic states for any genotype-environment interaction depends on how well the genotype and environmental factors are characterized.

The question, then, is which of these feasible metabolic states is manifested in the biological system under consideration. FBA postulates that the metabolic system exhibits a metabolic state that is optimal under some criteria. This objective is expressed as a linear combination of the fluxes contained in \(v\). The model can then be formulated as a linear programming problem as follows

\[
\begin{align*}
\text{minimize (maximize)} \quad & Z = \sum_i c_i v_i \\
\text{such that} \quad & S \cdot v - b = 0, \quad 0 \leq \beta_i \leq v_i \leq \alpha_i
\end{align*}
\]

\(Z\) is the objective function, representing a phenotypic property, and \(c\) is a vector of weights that are either costs of or benefits derived from the fluxes. The limits, \(\alpha_i\) and \(\beta_i\), represent known constraints on the maximum or minimum values that the fluxes assume. When the maximization of ATP production is considered, the net flux of ATP hydrolysis (\(v_{ATP\_PR}\)) is maximized.

\[
\text{maximize } Z = v_{ATP\_PR}
\]

The balance equations expressed by \(equation\ 1\) form part of the constraints. A commercially available package was used to solve the LP problems (Lindo Systems, Chicago, IL). The objective \(Z\) is minimized or maximized, subject to the imposed constraints. There are two parameters associated with the linear programming (LP) problem that help characterize the optimal solution as well as help in post-optimality analysis. These are the shadow price (\(\gamma_i\)) and the reduced cost. The \(\gamma_i\) are associated with each metabolite and are defined as

\[
\gamma_i = \frac{dZ}{d \Phi_i}
\]

For the objective of maximizing ATP production, if the \(\gamma_i\) of NADH is 3.00, it means that an additional molecule of NADH can generate three more molecules of ATP. The \(\gamma_i\), therefore, represents the increase in the value of the objective with the addition of the associated intermediate. The reduced costs are associated with each flux (\(v_i\)) and signify the amount by which the objective function is decreased if \(v_i\) is brought into the basis solution. For instance, if the input flux of lactate shows a reduced cost of 17.5, it means that increasing that flux by one unit will increase ATP production by 17.5 units. Reduced costs and shadow prices are terms commonly used in translating LP solutions to real-life situations and carrying out an analysis of alternate solutions from the original solution. We introduce these quantities to analyze the physiological task of energy metabolism to gain insight into the solutions as well as provide a “microeconomic” perspective to the ATP-generation problem.

The mitochondrial model. The flux balance model for mitochondria presented here comprises the glycolytic pathways, TCA cycle, and the electron transport system (ETS). The pentose phosphate pathway has not been included, because its activity is believed to be quite low for mitochondria-related functions (19). The oxidative metabolism of substrates takes place in the mitochondria; thus the substrates, metabolites, and cofactors must cross the selectively permeable membrane that separates the mitochondrial space from the cytosolic space. The reactions comprising the TCA cycle occur in the mitochondrion and produce the reduced coenzymes NADH and FADH\(_2\) that transfer electrons to oxygen in a regulated manner. Shuttles play an important role in transporting reducing equivalents generated in the cytosol into the mitochondrion.

The reactions, which make up the model, are divided into three sets, based on whether they occur in the cytosol or in the mitochondria or in transporting an intermediate across the mitochondrial membrane. The enzymes considered in the
FBA model and their E.C numbers are listed in Table 1. The glycolytic reactions take place in the cytosol. The reactions in the TCA cycle and the ETS take place in the mitochondrial matrix. This compartmentalization of reactions in an organelle leads to the need for special reaction sequences, or shuttles, to transport reducing equivalents generated in the cytosol into the mitochondria. There are two such shuttles that have been found to be active in mitochondria.

The ubiquitous malate-aspartate shuttle transports external NADH into the mitochondria. The coordinated exchange of malate and \( \alpha \)-ketoglutarate with aspartate and glutamate by respective antiporters is a key feature of the shuttle (19, 24). The malate dehydrogenase reaction functions in opposite directions in the cytosol and in the matrix. The cytosolic reaction forms malate from oxaloacetate and involves the oxidation of NADH. Malate is then transported into the matrix and through the matrix with concomitant efflux of \( \alpha \)-ketoglutarate. Malate is used to form oxaloacetate in the matrix, and NADH is formed in this reaction. The cycle is completed by the transamination reactions involving oxaloacetate, \( \alpha \)-ketoglutarate, glutamate, and aspartate. The normal functioning of this shuttle is outlined in Fig. 1.

An alternate shuttle that occurs in the skeletal muscle and brain is the glycerol-3-phosphate shuttle (8). Here, the reducing equivalents from NADH are delivered as FADH\(_2\) into the mitochondria. Thus the energy yield is reduced compared with the malate-aspartate shuttle, because each FADH\(_2\) produces only 2 ATP compared with 3 ATP for NADH. There are two dehydrogenases involved in this shuttle, the cytosolic and mitochondrial glycerol-3-phosphate dehydrogenase. The cytosolic dehydrogenase reduces dihydroxyacetone phosphate to glycerol-3-phosphate using NADH, and the mitochondrial dehydrogenase mediates the reverse reaction, which generates FADH\(_2\).

Additionally, fluxes for substrate input and intermediate secretion are also included in the model. The input fluxes for the substrates can be constrained for the different situations that will be studied.

The postulated objective for mitochondrial energy metabolism. The flux-balance model as explained earlier, requires an objective, which the cell or the organelle, as is the case here, is attempting to achieve. This objective is generally a postulated theoretical assumption that completes the model formulation and enables us to simulate metabolic behavior. For this analysis, maximizing the production of ATP is chosen as the objective function. Therefore, a single flux that hydrolyses the net ATP produced was considered, and the objective function was mathematically represented as \( v_{\text{ATP\_USE}} \), with a corresponding cost of unity. Cairns et al. (4) considered the functional basis for control of mitochondrial oxidative phosphorylation in different organs for rat mitochondria. ATP production in the brain and heart mitochondrial systems was found to use more oxygen but produce ATP at a faster rate than liver systems. They attribute these qualities to the thermodynamic degree of

**Table 1. The fluxes in the FBA model**

<table>
<thead>
<tr>
<th>Enzyme/Input Reaction</th>
<th>E.C. Number</th>
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<th>E.C. Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose input</td>
<td></td>
<td>Citrate synthase</td>
<td>4.1.3.7</td>
</tr>
<tr>
<td>Oxygen input</td>
<td></td>
<td>Pyruvate dehydrogenase</td>
<td>1.2.4.1</td>
</tr>
<tr>
<td>Lactate input</td>
<td></td>
<td>Acetate</td>
<td>4.2.1.3</td>
</tr>
<tr>
<td>Palmitate output</td>
<td></td>
<td>Isocitrate dehydrogenase</td>
<td>1.1.1.42</td>
</tr>
<tr>
<td>Lactate drain</td>
<td></td>
<td>( \alpha )-Ketoglutarate dehydrogenase</td>
<td>1.2.4.2</td>
</tr>
<tr>
<td>Oxaloacetate drain</td>
<td></td>
<td>Succinate dehydrogenase</td>
<td>1.3.99.1</td>
</tr>
<tr>
<td>Hexokinase</td>
<td>2.7.1.2</td>
<td>Fumarate reductase</td>
<td>1.3.99.1</td>
</tr>
<tr>
<td>Phosphoglucomiase</td>
<td>5.3.1.19</td>
<td>Fumarase</td>
<td>4.2.1.2</td>
</tr>
<tr>
<td>Phosphofructokinase</td>
<td>2.7.1.11</td>
<td>Malate dehydrogenase (isozyme)</td>
<td>1.1.1.27</td>
</tr>
<tr>
<td>Fructosebisphosphatase</td>
<td>3.1.3.11</td>
<td>NADH dehydrogenase (complex I)</td>
<td>1.6.5.3</td>
</tr>
<tr>
<td>Aldolase</td>
<td>4.1.2.13</td>
<td>Ubiquinone cytochrome c-oxidoreductase</td>
<td>1.10.2.2</td>
</tr>
<tr>
<td>Triose-P-isomerase</td>
<td>5.3.1.11</td>
<td>Cytochrome c-oxidase</td>
<td>1.9.3.1</td>
</tr>
<tr>
<td>Glyceraldehyde-3-phosphate</td>
<td>1.2.1.12</td>
<td>ATP synthase</td>
<td>3.6.1.34</td>
</tr>
<tr>
<td>dehydrogenase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphoglycerate kinase</td>
<td>2.7.2.3</td>
<td>Palmitate thiokinase</td>
<td>6.2.1.3</td>
</tr>
<tr>
<td>Phosphoglycerate mutase</td>
<td>5.4.2.1</td>
<td>Acetyl-CoA dehydrogenase</td>
<td>1.3.99.3</td>
</tr>
<tr>
<td>Enolase</td>
<td>4.2.1.11</td>
<td>Acetyl-CoA acetyltransferase</td>
<td>2.3.1.16</td>
</tr>
<tr>
<td>PEP kinase</td>
<td>2.7.1.40</td>
<td>3-Hydroxyacyl-CoA dehydrogenase</td>
<td>1.1.1.35</td>
</tr>
<tr>
<td>PEP synthase</td>
<td>2.7.9.2</td>
<td>Acetyl-CoA acetyltransferase</td>
<td>2.3.1.16</td>
</tr>
<tr>
<td>PEP carboxy kinase</td>
<td>4.1.1.49</td>
<td>Aspartate aminotransferase</td>
<td>2.6.1.1</td>
</tr>
<tr>
<td>PEP carboxylase</td>
<td>4.1.1.31</td>
<td>Cytochrome 3-P-3-dehydrogenase</td>
<td>1.1.1.8</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td>1.1.2.4</td>
<td>Mitochondral glycerol-3-P-dehydrogenase</td>
<td>1.19.5.5</td>
</tr>
<tr>
<td>Malate-( \alpha )-ketoglutarate exchange</td>
<td></td>
<td>Glutamate-aspartate exchange</td>
<td></td>
</tr>
</tbody>
</table>

PEP, phosphoenolpyruvate; FBA, flux-balance analysis.

Fig. 1. The normal role of the malate-aspartate shuttle in mitochondria. OAA, oxaloacetate.
oxidative coupling. The general conclusion is that maximizing the rate of energy production, rather than maintaining thermodynamic efficiency, is the important characteristic of mitochondria in physiological systems. This conclusion supports the postulate that the objective of mitochondrial metabolism is the maximization of ATP production. This postulate requires several detailed studies to be experimentally validated. In this modeling study, its role is to provide a complete theoretical framework to draw insights into the constraints imposed by stoichiometry on energy metabolism.

RESULTS

Substrate preferences. The flux distributions for optimal production of ATP, shown in Fig. 2, A-C, were determined under conditions in which the substrate uptake is restricted to 1 mol·unit time$^{-1}$·unit mass dry wt$^{-1}$ or one unit. The simulations were carried out for the three substrates, glucose, lactate, and palmitic acid. Table 2 lists the constraints imposed on the different fluxes. The complete utilization of 1 mol of glucose results in the formation of 38 ATP with the concomitant utilization of 6 mol of O$_2$. The utilization of 1 mol of lactate forms 17.5 ATP with the utilization of 3.0 mol of O$_2$, and the utilization of palmitic acid (a 14-C fatty acid) produces 129 ATP but requires 23 mol of O$_2$. Glucose is the preferred substrate compared with lactate and 14-carbon fatty acid when the oxygen flux is restricted and all three substrates are made available. Because glucose uptake produces maximum ATP per mole of oxygen consumed, ATP synthesis from glucose is the optimal strategy for energy metabolism.

Table 3 compares the three substrates in terms of the ATP yield on a per-oxygen basis and a per-carbon basis. Whereas the fatty acid provides more ATP on a per-carbon basis, the cost in terms of oxygen uptake makes the fatty acid a suboptimal alternative to the other substrates. The reduced costs contain information on the contribution of each flux to the optimal solution. The input fluxes for lactate and for fatty acid reflect reduced costs of 17.5 and 129.00, respectively, when glucose is the energy source. Because this is the number of ATP that can be synthesized from either substrate, these fluxes represent an alternate option for ATP synthesis. Therefore, if the oxygen uptake flux increases by 3 units and the glucose uptake flux remains constant, the uptake of 1 unit of lactate will yield an additional 17.5 units of ATP.

The malate-aspartate shuttle is active when glucose or lactate is the substrates for energy metabolism. When glycolysis is active, the glycerol-3-phosphate shuttle is a secondary option for transporting reducing equivalents into the mitochondrion from the cytosol. This exchanges one molecule of cytosolic NADH for one mitochondrial FADH$_2$ and is therefore an inferior op-

Fig. 2. The optimal metabolic flux distributions for the maximal production of ATP from glucose (Glu; A), lactate (B), and palmitate (C). The figures have been simplified by representing linear sequences of fluxes as a single flux and only showing the key branch points. The cytosolic and mitochondrial metabolites have been identified by the letters c and m, respectively. The malate-aspartate shuttle is active for the metabolism of lactate and glucose, and there is an efflux of α-ketoglutarate (AKG) out of the mitochondrion accompanied by an influx of malate (Mal). PEP, phosphoenolpyruvate; ETS, electron transport system.
tion and oxygen consumption are completely balanced. In region B, the total carbon flux is in stoichiometric excess. Glucose is completely consumed, and the palmitate consumption corresponds to the appropriate flux on the boundary region.

In normal functioning hearts under aerobic conditions, free fatty acids (FFA) are primarily used for energy production. FBA predicts that under these circumstances, the ideal substrate to maximize ATP production is glucose. However, it is known that FFA metabolism inhibits pyruvate dehydrogenase (PDH) and phosphofructokinase (PFK) activities and reduces the flux of glucose catabolism. This metabolic regulation can be explicitly modeled in the FBA model, in addition to the stoichiometric requirements, by constraining the fluxes through these enzymes. Therefore, when PDH and PFK are inhibited, the primary source for energy is through FFA metabolism. From the phase plane (Fig. 3C), it is evident that if the glucose flux were constrained to be lower than the stoichiometric maximum of 1 unit for complete oxidation, the consumption of palmitate would increase. If the glucose flux were at 0.2, because of enzyme inhibition, the corresponding flux of palmitate for complete oxidation would be relatively close to the stoichiometric maximum and hence the energy derived from palmitate would be significantly higher than that from glucose.

Altering enzyme activity. The FBA model can be used to evaluate the systemic consequences of reduced enzyme activity. Mathematically, this is equivalent to setting a constraint on a specific flux (e.g., $v_{\text{AKGDH}}$) to be equal to 0 or some specific value, i.e., $\alpha_i = \beta_i = 0$ or some specific value. The effect of alterations in the activity of different enzymes involved in the metabolism of ATP production was investigated for the different substrates. Table 2 details the constraints imposed for the following scenarios.

Glucose as substrate. When citrate synthase, aconitase, or isocitrate dehydrogenase are inactive, the ATP yield drops significantly and the model predicts the accumulation of oxaloacetate. The predicted flux distribution for these cases is shown in Fig. 4A. The glucose input flux was maintained at 1 unit to compare with a normally functioning network. The carbon flux is partially cycled through phosphoenolpyruvate carboxylase and PDH. Figure 4B shows the dependence of ATP production on the activity of these enzymes. This dependence is linear, and the qualitative metabolic map is constant in this range of enzyme activities. That is, the active fluxes involved in energy metabolism remain the same until the enzyme activity is zero. An

<table>
<thead>
<tr>
<th>Enzyme/Input Reaction</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose input</td>
<td>1 0 0 1 1 1</td>
</tr>
<tr>
<td>Lactate input</td>
<td>0 1 0 0 0 0</td>
</tr>
<tr>
<td>Palmitate output</td>
<td>0 0 1 0 0 0</td>
</tr>
<tr>
<td>Citrate synthase</td>
<td>0</td>
</tr>
<tr>
<td>Aconitase</td>
<td>0</td>
</tr>
<tr>
<td>Isocitrate dehydrogenase</td>
<td>0</td>
</tr>
<tr>
<td>α-Ketoglutarate dehydrogenase</td>
<td>0</td>
</tr>
<tr>
<td>Succinyl-CoA synthetase</td>
<td>0</td>
</tr>
<tr>
<td>Succinate dehydrogenase</td>
<td>0</td>
</tr>
<tr>
<td>Fumarase</td>
<td>0</td>
</tr>
<tr>
<td>Malate dehydrogenase (isozyme)</td>
<td>3 0</td>
</tr>
</tbody>
</table>

Table 2. Constraints on key fluxes for the model simulations

<table>
<thead>
<tr>
<th>Substrate</th>
<th>ATP Yield per Oxygen</th>
<th>ATP Yield per Carbon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>6.33</td>
<td>6.33</td>
</tr>
<tr>
<td>Lactate</td>
<td>5.83</td>
<td>5.83</td>
</tr>
<tr>
<td>Palmitic acid</td>
<td>5.61</td>
<td>9.21</td>
</tr>
</tbody>
</table>
interesting feature that is predicted in this situation is the secretion of oxaloacetate, resulting from the excess carbon flux through glycolysis entering the TCA cycle.

When any of the enzymes from α-ketoglutarate dehydrogenase to fumarase is inactive, the TCA cycle is again not completely functional. Excess carbon in this situation is secreted as α-ketoglutarate. An excess of α-ketoglutarate has in fact been identified as a feature of mitochondrial diseases involving mutations in the genes that code for these enzymes (12). The ATP yield is again significantly lower than the optimal value. The flux distribution for this scenario is shown in Fig. 5A. The dependence of ATP production on the activity of these enzymes is also linear and is shown in Fig. 5B.

The activity of the malate dehydrogenase enzyme also affects the ATP production in an adverse manner and predicts the accumulation of α-ketoglutarate. Interestingly, the effect of malate dehydrogenase is limited until the metabolic flux activity is reduced to <50% of the normal activity. The flux distributions predicted in this situation are different depending on the activity. Figure 6A shows the flux distribution...
when the malate dehydrogenase flux is 75% active. Here, the glycerol-3-phosphate shuttle is active and picks up the slack in malate-aspartate shuttle activity. Thus the reducing equivalents are shuttled by the glycerol-3-phosphate shuttle that exchanges a cytosolic NADH for a mitochondrial FADH₂. This results in a lower stoichiometric yield of ATP. Malate dehydrogenase is active in the cytosol as well as the mitochondria (18), and a loss in the activity of either or both isozymes leads to the same predicted metabolic state. When the malate dehydrogenase activity is zero, the flux distribution (Fig. 6B) shows that the malate-aspartate shuttle functions in the reverse direction compared with the normal case and the ATP yield is decreased. The dependence of ATP production on enzyme activity is therefore biphasic (Fig. 6C), and the slope of the linear dependence is steeper when malate dehydrogenase activity is <50%.

Table 4 lists the differences in ATP yield on oxygen carbon and carbon during energy metabolism from glucose. The ATP/carbon drops significantly, because most of the carbon is excreted out as organic acids, thus being incompletely metabolized.

Palmitate and lactate as substrates. When palmitate or lactate is the substrate, there is no ATP generation when any of the enzymes are fully inactive. During ATP generation from glucose, the anaplerotic reactions

![Diagram](image1)

Fig. 4. A: the predicted optimal flux distribution when citrate synthase, aconitase, or isocitrate dehydrogenase is inactive. The PEP carboxylase is active, and the carbon flux is shunted through this pathway into the incomplete tricarboxylic acid cycle (TCA) cycle. B: the dependence of ATP production on the activity of citrate synthase, aconitase, or isocitrate dehydrogenase.

![Diagram](image2)

Fig. 5. A: the predicted optimal flux distribution when AKG dehydrogenase, succinyl-CoA synthetase, succinate dehydrogenase, and fumarase are inactive. The carbon flux is partially shunted through this pathway and partially through pyruvate dehydrogenase into the incomplete TCA cycle. B: the dependence of ATP production on the activity of AKG dehydrogenase, succinyl-CoA synthetase, succinate dehydrogenase, or fumarase.
that are part of the TCA cycle are able to reroute the metabolites and provide some reduced cofactors for the ETS. This is not possible when lactate or palmitate is the substrate, because these substrates are directly converted into TCA cycle intermediates.

DISCUSSION

Mitochondrial function is a critical component of cellular physiology, and its metabolic objectives are constrained by stoichiometry. In this contribution, we have 1) constructed a model for mitochondrial energy metabolism under the stoichiometric constraints inherent in the biochemical reaction network, 2) applied the model to characterize the ATP production from three common substrates, and 3) simulated the effect of genetic changes on mitochondrial energy metabolism.

The network of biochemical reactions and metabolites that constitute the critical elements of mitochondrial function were identified. The steady-state mass balances on each metabolite were mathematically represented as an underdetermined matrix equation, \( S \times v = 0 \), where the vector \( v \) represents the set of all feasible fluxes satisfying the stoichiometric constraints. To find a unique solution, it was postulated that the role of mitochondrial metabolism is the maximization of ATP production. Mathematically, the model is then formulated as an LP problem with this objective and the stoichiometric constraints. By adding additional constraints to the
problem, which specify input fluxes for the different substrates, the LP can be solved to identify a unique flux distribution.

The model was applied to predict the maximal yield of ATP from the three common substrates of mitochondrial metabolism, glucose, lactate, and palmitate. The model predicts the expected ATP yields for each. The activities of the shuttles are also predicted in accordance with their accepted roles. The malate-aspartate shuttle is the preferred shuttle to transfer reducing equivalents across the mitochondrial membrane. The glycerol-3-phosphate shuttle is a secondary option, and it is also a less optimal route to synthesize ATP.

The model predictions are in qualitative agreement with key features of shuttle operation. Safer et al. (13) showed that amino-oxyacetate, which inhibits transaminase reactions, is less effective in inhibiting the transport of reducing equivalents into mitochondria with glucose than with lactate as substrate in rat myocardium. With the inhibition of the malate-aspartate shuttle, the glycerol-3-phosphate shuttle was found to allow a nearly equivalent rate of redox transfer across the mitochondria. Additionally, the flux of α-ketoglutarate to malate is equal to the rate of acetyl-CoA entry into the TCA cycle, which is also predicted by FBA (Fig. 2A).

The mitochondrial model was used to simulate the effect of changes in enzyme activities. Mutations in TCA cycle enzymes, particularly fumarase, α-ketoglutarate dehydrogenase, and succinate dehydrogenase have been implicated in mitochondrial diseases (3, 12). These diseases have been characterized by the accumulation of intermediates of the TCA cycle, primarily the presence of α-ketoglutarate and lactate. The result of loss of activity was investigated for all the TCA enzymes, and it was observed that when the enzymes in the series of reactions leading up to α-ketoglutarate were inactive, the ATP yield was very low and excess oxaloacetate was produced. When the enzymes in the second span of the TCA cycle, from α-ketoglutarate dehydrogenase to malate dehydrogenase, are inactive, the model predicts a low yield of ATP and the excess production of α-ketoglutarate. This prediction is in qualitative agreement with the data presented by Rustin et. al (12). They have observed that anomalous urinary excretion of specific organic acids, particularly α-ketoglutarate, occurs in patients with enzyme defects affecting the TCA cycle.

The pathogenesis of mitochondrial diseases can be very complex, owing to the variety of inheritance patterns and the diversity of metabolic states (23). Mutations can lead to different metabolic states and different mutations can produce similar metabolic states. Models can play a role in understanding and characterizing these complex relationships. The model presented here provides a platform on which more biochemical networks can be overlaid and other processes in which mitochondrial function plays a role can be studied.

The production of reactive oxygen species (ROS) as a result of defects in electron transport can lead to processes that activate the mitochondrial permeability transition pore and initiate a sequence of events that ultimately leads to cell apoptosis (7). The incorporation of the biochemical networks involved in this process in the formulation of an extended FBA model is being considered.

Wallace (23) states that the phenotypic expression of mtDNA diseases may involve two factors: the predisposing mutation and an age-related factor that causes a decline in mitochondrial function, which exacerbates the inherited defect. In terms of the biochemistry, this can be viewed as a change in the constraints of key fluxes over time, which can lead to qualitative and quantitative changes in the metabolic state. FBA has the necessary capabilities to address these issues. Flux analysis models themselves are not dynamic representations of metabolic functions. However, they provide the means to obtain several static snapshots of metabolism based on steady-state flux activity. Therefore, if a certain allosteric effect is known to effect an enzymatic reaction, the outcome of the severity of the effect on the metabolic function can be characterized. This can help complement a dynamic representation of the same effect that factors in substrates and product concentrations and their effect on the enzyme flux.

### Perspectives

This paper presents a model based on a systems-analysis approach to characterize the integrated energy metabolism of mitochondria. The model is completed by postulating that the objective of mitochondrial energy metabolism is to maximize the rate of ATP production. Such a postulate requires several experimental studies to be verified. However, the predictions from the postulated model can be used to drive such experiments. The model can incorporate genomic information to predict the combined effect of genotype changes and environmental perturbations on the metabolic state. For a well-characterized genotype, under certain environmental conditions, it is possible to determine metabolic states that represent all possible physiological outcomes. This paper explored the metabolic states related to mitochondrial energy metabolism under certain conditions. The model accurately predicts different scenarios of mitochondrial growth on the standard substrates. The model also is in qualitative agreement with studies on the physiology of mitochondrial dis-

<table>
<thead>
<tr>
<th>Enzyme Defect</th>
<th>ATP Yield per Oxygen</th>
<th>ATP Yield per Carbon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrate synthase, aconitase, isocitrate dehydrogenase, α-Ketoglutarate dehydrogenase, succinyl-CoA synthetase, succinate dehydrogenase, fumarase</td>
<td>6.5</td>
<td>2.17</td>
</tr>
<tr>
<td>Malate dehydrogenase</td>
<td>6.17</td>
<td>6.17</td>
</tr>
<tr>
<td></td>
<td>5.5</td>
<td>5.83</td>
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</table>
eases, particularly in the accumulation of TCA cycle intermediates. Flux analysis of mitochondrial metabolism promises to be a useful methodology to understand and characterize the pathophysiology of mitochondrial diseases. Mitochondrial function is a critical part of the physiology of several organs. The genetic defects in several enzymes can contribute to physiological diseases that manifest at the organ level. Flux analysis can link genetics and physiology by representing individual fluxes that are related to the action of a gene or a set of genes in a model and characterizing the overall flux distribution that is representative of the physiological response. The work is currently being extended to study further disease-related questions, particularly those involved in ROS formation.

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Present addresses: R. Ramakrishna, Physiome Sciences, Inc., 307, College Road East, Princeton, NJ 08540; J. S. Edwards, Dept. of Chemical Engineering, University of Delaware, Newark, DE 19716.

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