Modeling the ATP Production in Mitochondria

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Abstract We revisit here the mathematical model for ATP production in mitochondria introduced recently by Bertram, Pedersen, Luciani, and Sherman (BPLS) as a simplification of the more complete but intricate Magnus and Keizer’s model. We identify some inaccuracies in the BPLS original approximations for two flux rates, namely the adenine nucleotide translocator rate $J_{\text{ANT}}$ and the calcium uniporter rate $J_{\text{uni}}$. We introduce new approximations for such flux rates and then analyze some of the dynamical properties of the model. We infer, from exhaustive numerical explorations, that the enhanced BPLS equations have a unique attractor fixed point for physiologically acceptable ranges of mitochondrial variables and respiration inputs, as one would indeed expect from homeostasis. We determine, in the stationary regime, the dependence of the mitochondrial variables on the respiration inputs, namely the cytosolic concentration of calcium $C_{a_c}$ and the substrate fructose 1,6-bisphosphate FBP. The same dynamical effects of calcium and FBP saturations reported for the original BPLS model are observed here. We find out, however, a novel nonstationary effect, which could be, in principle, physiologically interesting: some response times of the model tend to increase considerably for high concentrations of calcium and/or FBP. In particular, the larger the concentrations of $C_{a_c}$ and/or FBP, the larger the necessary time to attain homeostasis.

Keywords Mitochondria · Calcium · ATP · Mathematical model
1 Introduction

The exchange of energy in cells is mostly mediated by ATP (adenosine triphosphate) molecules. Such molecules are produced in several processes in an eukaryotic cell, but the principal source of ATP is typically the oxidative phosphorylation process, which takes place in mitochondria. The mitochondrion is an organelle with two membranes, having, therefore, two distinct bulk regions: the intermembrane space and the mitochondrial matrix. In the inner membrane, there are plenty of protein transporters and ionic channels, some of which execute an active transport leading to a gradient of some ions and molecules (Guyton and Hall 2006; Nelson and Cox 2004). The metabolic cascade that leads to the production of ATP in the mitochondrion starts in the cytoplasm. At first, glucose is transported from the extracellular medium into the cytoplasm by GLUT transporters. It is then converted in glucose-6-phosphate (G6P) by the enzyme hexokinase. G6P is then converted in pyruvate in a process called glycolysis, in which there is a net production of two ATP molecules. The pyruvate produced is transported into the mitochondrion (to the mitochondrial matrix) and is metabolized in a series of oxidation-reduction reactions in the citric acid cycle leading to the production of the nicotinamide adenine dinucleotide NAD and flavin adenine dinucleotide FAD. These electron donor molecules are oxidized in the complexes I to IV present in the inner mitochondrial membrane. These reactions lead to the activation of a proton pump, creating a pH gradient between the inter membrane space and the matrix. The protons pumped into the intermembrane space return to the matrix through a transporter that uses their energy to catalyze the conversion of ADP (adenosine diphosphate) into ATP. The ATP produced in the mitochondria is then transported to the cytoplasm by the ATP/ADP exchanger (Guyton and Hall 2006; Nelson and Cox 2004).

The kinetic aspects of the processes involved in the ATP production in mitochondria are rather intricate. This issue was addressed by Magnus and Keizer (MK), who introduced in the series of papers (Magnus and Keizer 1997, 1998a, 1998b) a theoretical kinetic model for ATP production in mitochondria based on the known biophysical properties of the enzymes and transporters involved in the process. In fact, the MK model was built by considering electrical activity and cytosolic calcium handling in insulin-secreting pancreatic β-cells. The model consists basically in a set of equations describing the dynamics of the citric acid cycle, the proton pump, and the inner mitochondrial membrane transporters of ATP and calcium. The MK model is effectively based on first biophysical principles and provides a very detailed and accurate description of the processes considered to be important for mitochondrial oxidative phosphorylation. However, it is also a rather complex model with cumbersome equations, preventing a systematic mathematical study of its main dynamical and physiological properties.

A simplification of the MK model aiming to retain its main dynamical properties was introduced recently by Bertram, Pedersen, Luciani, and Sherman (BPLS) in Bertram et al. (2006). The BPLS model incorporates some refinements introduced by Cortassa et al. (2003) for the description of the ATP production in cardiac cells. In fact, BPLS model can be considered as an approximation of the Cortassa et al.’s model instead of the original MK one. As we will see, this was probably the origin of some inaccuracies in the BPLS equations. As in the original MK model, the
mitochondrial ATP production in the BPLS model is governed by four dynamical variables, namely the potential drop in the inner membrane $\Delta V$ and the mitochondrial concentrations of: reduced nicotinamide adenine dinucleotide NADH, adenosine diphosphate ADP, and calcium $C_{a_m}$. The mitochondrial concentrations of pyridine and adenine nucleotides are assumed to be conserved

$$NAD_m + NADH_m = NAD_{tot},$$

$$ADP_m + ATP_m = A_{tot},$$

where $NAD_{tot}$ and $A_{tot}$ stand for the total mitochondrial concentration of the respective nucleotides. The balance of the pertinent fluxes and reactions yields to the following dynamical equations for the mitochondrial variables:

$$\frac{d}{dt}NADH = J_{PDH} - J_0,$$

$$\frac{d}{dt}ADP = J_{ANT} - J_{F1F0},$$

$$\frac{d}{dt}C_{a_m} = f_m(J_{uni} - J_{NaCa}),$$

$$\frac{d}{dt}\Delta V = C_{m}^{-1} (J_H - J_{ANT} - J_{NaCa} - 2J_{uni}),$$

where

$$J_H = J_{H, res} - J_{H, ATP} - J_{H, leak}.$$  

The derivation and meaning of the fluxes presented in the right-handed sides of Eqs. (3)–(6) are rather involved. The main details and the pertinent references can be found, for instance, in the BPLS paper (Bertram et al. 2006). We have checked carefully the derivation of each of these fluxes and we have found out some inaccuracies in the BPLS expressions for the adenine nucleotide translocator rate $J_{ANT}$ and for the calcium uniporter rate $J_{uni}$. As we will see, some of these problems probably have originated in the transcription of the original MK equations to the Cortassa et al.’s model.

In the present paper, we propose some enhanced approximations in the BPLS framework for the fluxes $J_{ANT}$ and $J_{uni}$ and analyze some of the dynamical properties of Eqs. (3)–(6). We show, in particular, that for physiologically acceptable ranges of mitochondrial respiration inputs, namely the cytosolic concentration of calcium $Ca_c$ and the substrate fructose 1,6-bisphosphate FBP, the BPLS equations have a unique physiologically acceptable attractor fixed point, as one would indeed expect for any model compatible with homeostasis. Exhaustive numerical explorations indicate that the BPLS model is indeed globally stable, reinforcing its relevance to physiological quantitative studies, despite its simplicity when compared to the MK original model. We determine, in the stationary regime, the dependence on constant respiration inputs $Ca_c$ and FBP of the four mitochondrial variables considered in the model. As in the original BPLS model, we observe here qualitatively distinct dynamical behavior for low and high concentrations of $Ca_c$ and/or FBP. We detect, moreover, a nonstationary
effect, which could be, in principle, physiologically interesting: the inertia of the system tends to increase considerably for high concentrations of cytosolic calcium and FBP, i.e., some response times of the model tend to increase considerably for high respiration inputs $C_{ac}$ and FBP. In particular, the larger the concentrations of $C_{ac}$ and/or FBP, the larger the necessary time to attain homeostasis.

### 2 The Enhanced BPLS Model

We will focus here in the problems we found for the BPLS expressions for the adenine nucleotide translocator rate $J_{\text{ANT}}$ and for calcium uniporter rate $J_{\text{uni}}$, since all the other quantities appearing in (3)–(6) were checked to be correct and accurate for physiological ranges of variables and parameters. The MK expression for the former is (see Eq. (16) and Table 4 of Magnus and Keizer 1997)

$$J_{\text{ANT}} = V_{\text{max,A NT}} \frac{1 - \frac{\alpha_c ATP_c ADP_m}{\alpha_m ATP_c ATP_m} e^{-\frac{F \Delta V}{RT}}}{(1 + \frac{\alpha_c ATP_c}{ADP_c} e^{-f \frac{F \Delta V}{RT}})(1 + \alpha_m^{-1} \frac{ADP_m}{ATP_m})}. \quad (8)$$

The precise meaning of all the quantities presented in this formula can be found in Magnus and Keizer (1997, 1998a, 1998b), and in the BPLS paper (Bertram et al. 2006) as well. (For the values of the parameters, see Table 1.) On the other hand, the expression for $J_{\text{ANT}}$ presented in the Eq. (35) of Cortassa et al. 2003 reads

$$J_{\text{ANT}} = V_{\text{max,A NT}} \frac{1 - \frac{\alpha_c ATP_c ADP_m}{\alpha_m ATP_c ATP_m}}{(1 + \frac{\alpha_c ATP_c}{ADP_c} e^{-f \frac{F \Delta V}{RT}})(1 + \alpha_m^{-1} \frac{ADP_m}{ATP_m})}. \quad (9)$$

By comparing with (8), we see clearly that it lacks the exponential in the numerator. Furthermore, the incorrect expression (9) is transcribed in the BPLS Eq. (35) as

$$J_{\text{ANT}} = V_{\text{max,A NT}} \frac{\frac{ATP_m}{ADP_m} - \frac{\alpha_c ATP_c}{\alpha_m ADP_c}}{(1 + \frac{\alpha_c ATP_c}{ADP_c})(\frac{ATP_m}{ADP_m} + \alpha_m^{-1}) e^{-f \frac{F \Delta V}{RT}}}, \quad (10)$$

i.e., with another mistake in the denominator. The BPLS expression for $J_{\text{ANT}}$, obtained from (10) after some simplifications, is

$$J_{\text{ANT}} = p_{19} \left( \frac{\frac{ATP_m}{ADP_m}}{\frac{ATP_m}{ADP_m} + p_{20}} \right) e^{f \frac{F \Delta V}{RT}}, \quad (11)$$

where $p_{19}$ and $p_{20}$ are some (fitted) numerical parameters. The (reasonable) physiological hypothesis used to derive (11) in the BPLS model is the assumption that, due to the ion transporters action, the rates of ATP to ADP in the mitochondrial matrix and in the cytoplasm are approximately the same,

$$\frac{ATP_c}{ADP_c} \approx \frac{ATP_m}{ADP_m}. \quad (12)$$
Note that this assumption implies from (8) that \( J_{\text{ANT}} \approx 0 \) for \( \text{ATP}_m \rightarrow \text{A}_{\text{tot}} \) (and, hence, \( \text{ADP}_m \rightarrow 0 \) according to (2)), which is incompatible with the BPLS expression (11). Another qualitatively different behavior arises for large values of \( \Delta V \): Eq. (8) implies that \( J_{\text{ANT}} \) tends to an asymptote, whereas (10) suggests an exponential growth. The expression (10) is clearly not accurate as an approximation of (8).

With the assumption (12) and taking into account the conservation of mitochondrial pyridine nucleotides (2), the original MK expression (8) for the adenine nucleotide transporter rate reads

\[
J_{\text{ANT}} = V_{\text{max,ANT}} \left( \frac{\text{ATP}_m}{\text{A}_{\text{tot}} - (1 - \alpha_m)\text{ATP}_m} \right) \frac{\alpha_m - \alpha_c e^{-\frac{F \Delta V}{RT}}}{1 + \alpha_c \frac{\text{ATP}_m}{\text{A}_{\text{tot}} - \text{ATP}_m} e^{-f \frac{F \Delta V}{RT}}}.
\]

(13)

This is our first proposed approximation, which captures all the essential properties of (8) and is still simple enough to be mathematically manipulated. Notice that for the typical range of physiological parameters, neglecting the exponential in the numerator of (13) would imply a relative error inferior to 5%. We will not, however, adopt this further approximation in this work. Figure 1 illustrates the discrepancies between the expressions (11) and (13) for typical physiological values of the parameters and variables. A closer inspection of the graphics (9) of Bertram et al. (2006) reveals that they have probably compared their approximated expression (11) with Eq. (10), which was itself transcribed incorrectly from Cortassa et al.’s Eq. (9).

With respect to the calcium uniporter rate \( J_{\text{uni}} \), the original MK expression reads (see Eq. (19) in Magnus and Keizer 1997)

\[
J_{\text{uni}} = V_{\text{max,uni}} \frac{\frac{2F}{RT} (\Delta V - \Delta V_0)}{1 - e^{-\frac{2F}{RT} (\Delta V - \Delta V_0)}} \left( \frac{\frac{C_{a_c}}{K_{\text{trans}}} (1 + \frac{C_{a_c}}{K_{\text{trans}}})^3}{(1 + \frac{C_{a_c}}{K_{\text{trans}}})^4 + \frac{L}{(1 + C_{a_c}/K_{\text{act}})^{\beta_a}}} \right).
\]

(14)

In the BPLS derivation of the approximation for \( J_{\text{uni}} \), it used Eq. (38) of Cortassa et al. 2003, which reads

\[
J_{\text{uni}} = V_{\text{max,uni}} \frac{\frac{C_{a_c}}{K_{\text{trans}}} (1 + \frac{C_{a_c}}{K_{\text{trans}}})^3 \frac{2F}{RT} (\Delta V - \Delta V_0)}{(1 + \frac{C_{a_c}}{K_{\text{trans}}})^4 + \frac{L}{(1 + C_{a_c}/K_{\text{act}})^{\beta_a}} (1 - e^{-\frac{2F}{RT} (\Delta V - \Delta V_0)})},
\]

(15)

where one can see that there is a mistake in the denominator. The BPLS proposed expression for the calcium uniporter rate, obtained as a simplification of (15), is

\[
J_{\text{uni}} = (p_{21} \Delta V - p_{22}) C_{a_c}^2,
\]

(16)

where \( p_{21} \approx 0.01 \text{mM}^{-1} \text{ms}^{-1} \text{mV}^{-1} \) and \( p_{22} \approx 1.1 \text{mM}^{-1} \text{ms}^{-1} \) are also fitted numerical parameters. We found this equation to be inaccurate for the typical physiological range of parameters as well (see Fig. 1). Notice, in particular, that it implies in nonpositive flux rates for \( \Delta V \leq p_{22}/p_{21} \approx 110 \text{ mV} \). We propose to keep in the approximated model the complete original MK equation (14). Its dependence on \( \Delta V \) is already in a rather simple form, and the complications for \( C_{a_c} \) are harmless for the dynamical studies, as we will show.
Fig. 1  Comparison between the original BPLS expressions and our proposals based on the original MK model. (Above) The BPLS adenine nucleotide translocator rate $J_{\text{ANT}}$ (11) and our proposal (13). We notice that the variation of (13) over physiological ranges (the inserted graphics) is considerable smaller than that one of (11). Furthermore, even the concavities of the curves are different. The dependency of (11) on $\Delta V$ is exponential, whereas (13) tends to an asymptote for large values of $\Delta V$. In accordance to Table 1, these curves were calculated by assuming ATP = 500 $\mu$M and $A_{\text{tot}}$ = 15 $\mu$M. (Below) The BPLS calcium uniporter rate $J_{\text{uni}}$ (16) and the original MK expression (14), both calculated for $C_{a_c}$ = 0.2 $\mu$M. Equation (16) is a straight line, which implies nonpositive rates for physiological values of $\Delta V$ (Color figure online)

For the dynamical analysis, it is more conveniently to introduce the following dimensionless variables:

$$x = \frac{NAD_{\text{m}}}{NAD_{\text{tot}}}, \quad (17)$$
$$y = \frac{ATP_{\text{m}}}{A_{\text{tot}}}, \quad (18)$$
$$z = \frac{C_{a_m}}{C_{a_0}}, \quad (19)$$
$$w = \frac{\Delta V}{\Delta V_0}, \quad (20)$$
Taking into account the new proposed expressions (13) and (14), the rates in the right-handed sides of (3)–6) will be given by

\begin{align*}
J_{\text{PDH}} &= r_1 \sqrt{\frac{v}{a_1 + z}} \left( a_2 + \frac{x}{1 - x} - 1 \right), \\
J_0 &= r_2 \frac{x}{a_3 + x} \left( 1 + a_4 e^{a_5 w} \right)^{-1}, \\
J_{\text{ANT}} &= r_3 \left( \frac{y}{1 - a_6 y} \right) \frac{a_7 - a_8 e^{-a_9 w}}{1 + a_8 \frac{y}{1 - y} e^{-a_9 w}} , \\
J_{F1F0} &= r_4 \left[ (a_{11} + y) \left( 1 + a_{12} e^{-a_{13} w} \right) \right]^{-1}, \\
J_{H, \text{res}} &= a_{14} J_0, \\
J_{H, \text{ATP}} &= a_{15} J_{F1F0}, \\
J_{H, \text{leak}} &= r_5 (w - a_{16}), \\
J_{\text{NaCa}} &= r_6 \frac{z}{u} e^{a_{17} w}, \\
J_{\text{uni}} &= r_7 \frac{a_{18}(w - 1)}{1 - e^{-a_{18}(w - 1)}} G(u),
\end{align*}

where

\begin{equation}
G(u) = \frac{u (1 + a_{19} u)^{a_4} (1 + a_{20} u)^3}{a_{21} + (1 + a_{19} u)^{a_4} (1 + a_{20} u)^4}.
\end{equation}

All the values of the numerical parameters and constants are presented in Table 1. With the new dimensionless variables, Eqs. (3)–(6) can be cast in the form

\begin{align*}
\dot{x} &= \frac{1}{\text{NAD}_{\text{tot}}} (J_{\text{PDH}} - J_0), \\
\dot{y} &= \frac{1}{A_{\text{tot}}} (J_{F1F0} - J_{\text{ANT}}), \\
\dot{z} &= \frac{f_m}{\text{Ca}_0} (J_{\text{uni}} - J_{\text{NaCa}}), \\
\dot{w} &= \frac{1}{C_m \Delta V_0} (J_H - J_{\text{ANT}} - J_{\text{NaCa}} - 2J_{\text{uni}}),
\end{align*}

where $f_m$ and $C_m$ stand, respectively, for the fraction of free Ca ions and the mitochondrial capacitance; see Table 1. Equations (33)–(36) form a non-autonomous systems of four first-order differential equations. The external excitations $u(t)$ and
\( \Delta V_0 = 91 \text{ mV} \)
\( V_{\text{max, ANT}} = 5 \mu M \text{ ms}^{-1} \)
\( f = 0.5 \)
\( L = 110 \)
\( a_c = 0.111 \)
\( r_1 = 0.2 \mu M \text{ ms}^{-1} \)
\( r_4 = 23.3 \mu M \text{ ms}^{-1} \)
\( r_7 = 0.11 \mu M \text{ ms}^{-1} \)
\( a_1 = 0.05 \)
\( a_4 = 4.23 \times 10^{-16} \)
\( a_7 = 0.139 \)
\( a_{10} = 1.68 \)
\( a_{13} = 10.7 \)
\( a_{16} = 0.16 \)
\( a_{19} = 0.52 \)
\( N_{\text{ADtot}} = 10 \times 10^3 \mu M \)
\( A_{\text{tot}} = 15 \times 10^3 \mu M \)
\( V_{\text{FBP}} = 1 \mu M \)
\( V_{\text{max, uni}} = 10 \mu M \text{ ms}^{-1} \)
\( K_{\text{trans}} = 19 \mu M \)
\( f_m = 0.01 \)
\( a_m = 0.139 \)
\( r_2 = 0.6 \mu M \text{ ms}^{-1} \)
\( r_5 = 0.182 \mu M \text{ ms}^{-1} \)
\( a_2 = 1 \)
\( a_5 = 18.2 \)
\( a_8 = 0.111 \)
\( a_{11} = 0.67 \)
\( a_{14} = 11.7 \)
\( a_{17} = 1.46 \)
\( a_3 = 0.01 \)
\( a_6 = 0.861 \)
\( a_9 = 3.37 \)
\( a_{12} = 5.10 \times 10^9 \)
\( a_{15} = 3.43 \)
\( a_{18} = 6.73 \)
\( a_{20} = 0.01 \)
\( a_{21} = 110 \)

\( v(t) \) are related, respectively, to the cytosolic concentration of calcium \( C_a \) and the substrate fructose 1,6-bisphosphate FBP; see Eqs. (21) and (22). We can now start the dynamical analysis of the model.

### 3 Dynamics of the Model

Let us consider initially the fixed points \((x_*, y_*, z_*, w_*)\) of the system (33)–(36) assuming constant inputs \((u_*, v_*)\). By construction, the physiologically meaningful range for the variables \(x\) and \(y\) is \([0, 1]\); see (1)–(2) and (17)–(18). For \(z\) and \(w\), we assume only that they are nonnegative. The typical physiological range for the potential drop, however, is more restrictive, corresponding to \(\Delta V \approx [90, 225] \text{ mV}\), which is equivalent to \(w \approx [1, 2.5]\). For the inputs \(u\) and \(v\), we consider the ranges \([0, 10]\) and \([0, 20]\), respectively, which corresponds to \(C_a \approx [0, 2] \mu M\) and \(\text{FBP} \approx [0, 20] \mu M\). We perform an exhaustive numerical search (Scilab files are available at [http://vigo.ime.unicamp.br/atp](http://vigo.ime.unicamp.br/atp)) for fixed points of (33)–(36) by assuming \(u \in [0, 10]\) and \(v \in [0, 20]\) constants. For all tested values of \(u\) and \(v\), only one physiological \((x, y \in [0, 1])\) both \(z, w > 0\) fixed point was found, which is always stable. Moreover, the fixed point is globally stable for physiological ranges of variables, meaning that any solution of (33)–(36) with reasonable initial conditions will tend asymptotically to the fixed point, i.e., the system indeed exhibits an asymptotic behavior compatible with homeostasis. Starting at a random point in the phase space, the variables \(w\) and \(x\) have typically the quickest convergence to the fixed point, where \(y\) and \(z\) are the slowest ones. The values of \((x_*, y_*, z_*, w_*)\) as function of the constant inputs \((u_*, v_*)\)
are depicted in Fig. 2, from where one can already observe some physiologically consistent dynamical properties which we describe in detail below.

The first observations is that the production of ATP and the concentration of NADH vanishes in the absence of cytosolic calcium $Ca_c$ and/or the substrate fructose 1,6-bisphosphate FBP, i.e., $x^* \rightarrow 0$ for $u^*$ or $v^* \rightarrow 0$. Notice that, from the condition $J_{NaCa} = J_{uni}$ defining the fixed point $\dot{z}^* = 0$ (see Eq. (35)), we have $z^* = 0$ for $u^* = 0$. On the other hand, $J_{PDH}$ vanishes for $z^* = 0$ (and for $v^* = 0$ as well), which implies via the condition $\dot{x}^* = 0$ that $J_0 = 0$ and, consequently, $x^* = 0$. The condition for $y^*$ and $w^*$ are more involved. The former vanishes for vanishing $u^*$ or $v^*$, while the latter will be given by $w^* \approx a_{16} = 0.16$ for $u^* = 0$. Also, we see that for reasonable values of $u^*$ and $v^*$ the value of the potential drop $\Delta V (w^*)$ is almost constant and close to 150 mV ($w^* = 1.65$). This stability is probably the reason why the original BPLS model is robust, despite the inaccuracies for the expression of $J_{ANT}$ and $J_{uni}$ we are correcting in this paper. We will return to this point in the last section. Still from the condition $J_{NaCa} = J_{uni}$, we see that $z^* \propto u^* G(u^*)$, since $w^*$ is almost constant for physiological reasonable values of $u^*$ and $v^*$ (see Fig. 2c).

Another important feature of the BPLS model is the reversion of the dynamical behavior of some mitochondrial variables in the presence of lower and higher concentration of cytosolic calcium and FBP. This behavior can be seen, for instance, in Fig. 2b. After attaining its maximum, the ATP production ($y^*$) tends to decrease for increasing cytosolic calcium concentrations ($u^*$). Calcium saturation can be simulated, as described in Bertram et al. (2006), by setting $a_1 = 0$ in the expression for
Fig. 3  Response of Eqs. (33)–(36) to oscillatory inputs (37). Notice that for low Ca concentrations (left), all the mitochondrial variables increases and decreases in synchrony with the variations of \( u \). On the other hand, for high Ca concentrations (right), the behavior of \( x \), \( y \) and \( w \) is reversed. All the curves were evaluated for FBP = 0.5 \( \mu \)M. See the text for further details.

\[ J_{\text{PDH}} \] (23). The reversion of the dynamical behavior of the other mitochondrial variables for higher Ca concentrations can also be inferred directly from Fig. 2, but it is certainly better illustrated in Fig. 3, which depicts the solutions of (33)–(36) for an oscillatory Ca input of the form

\[ u(t) = u_0 + u_1 \sin(t/t_0), \]  

(37)
with constant \( v(t) \) and initial conditions \((x(0), y(0), z(0), w(0))\) given by the values of the fixed point corresponding to \( u_\ast = u(0) \) and \( v_\ast = v(0) \). As we will see, such a choice of initial condition is consistent with the adiabatic (stationary) regime we observe for sufficiently slow inputs (large periods \( t_0 \)). For lower values of \( u_0 \) (low \( C_{ac} \) concentrations), all the mitochondrial variables increases and decreases in synchrony with the variations of \( u \). On the other hand, for higher values of \( u_0 \), the dynamical behavior of \( x \), \( y \) and \( w \) is reversed, i.e., they tend to decrease/increase while \( u \) increases/decreases. This effect can be understood from the relation between \( u_\ast \) and \( z_\ast \) depicted in Fig. 2c. The value of \( z_\ast \) tends to increase rapidly when \( u_\ast \) increases and, for large values of \( z \), the dependence of the expression for \( J_{PDH} \) on \( z \) saturates and becomes equivalent to setting \( a_1 = 0 \). Without the \( z \) suppression term in \( J_{PDH} \), the dynamical behavior of the variables \( x \), \( y \), and \( w \) is reversed, as it was pointed out in the original BPLS analysis. Low variations of \( v \) (FBP) do not change qualitatively this dynamical behavior. However, the situation changes for large concentrations of FBP. As described in Bertram et al. (2006), for low concentrations of FBP, the \( NADH_m \) concentration reacts to a sudden rising of \( C_{ac} \) with an upward teeth, while for high concentrations of FBP such behavior is reversed, i.e., \( NADH_m \) concentration exhibits a downward teeth if \( C_{ac} \) increases. This situation is analyzed and depicted in Fig. 4.

The oscillatory excitations used in the examples depicted in Figs. 3 and 4 have period \( t_0 = 3 \) min. For inputs varying over a time scale of minutes, the system evolves adiabatically in a good approximation, i.e., the instantaneous solution \((x(t), y(t), z(t), w(t))\) is well approximated by the fixed point \((x_\ast, y_\ast, z_\ast, w_\ast)\) corresponding to \( u_\ast = u(t) \) and \( v_\ast = v(t) \). In other words, for slowly varying inputs, the solutions of the system are confined to the fixed-point surfaces depicted in Fig. 2. Of course, one expects a breakdown of this adiabatic behavior for rapidly varying inputs. Nonstationary effects must appear for inputs varying with a characteristic time smaller than a certain critical value. In order to study nonstationary effects in our model, we consider the response of the system for inputs of the type

\[
u(t) = u_0 + u_1 \tanh\left(\frac{t - t_1}{t_0}\right),\tag{38}\]

for different values of \( t_0 \). This situation is depicted in Fig. 5 for some values of \( u_0 \) and \( u_1 \) and for \( t_0 = 2.5, 1, 0.5 \), and 0.02 s. It is clear that for lower values of \( u \) (\( C_{ac} \)), approximately 10 s are enough to assure that \( NADH_m \) concentration and \( \Delta V \) reaches their values corresponding to the adiabatic regime, which in this case corresponds to the homeostasis. As we have already noticed, the variables \( y \) (ATP) and \( z \) (\( C_{am} \)) are the slowest ones to attain their respective stationary regimes. For lower values of \( u \) (\( C_{ac} \)), they spend approximately 40 s to stabilize. Increasing the values of \( u \) implies the increasing of such “relaxation” times, i.e., a larger time is necessary to attain homeostasis. The second column in Fig. 5 corresponds to a situation with \( u \in [2, 4] \), for which almost 30 s are necessary to assure the attainment of the stationary regime for the rapid variable \( w \), whereas the slow one will need a few minutes. The inertia of the system, hence, increases considerably for higher concentrations of cytosolic calcium.

Higher concentrations of fructose 1,6-bisphosphate FBP also imply an increasing of the inertia of the system. This situation is analyzed and depicted in Fig. 6. Besides
Fig. 4  Response of Eqs. (33)–(36) to oscillatory inputs (37) for different concentrations of FBP. The system is submitted to the oscillatory input corresponding to the top graphic. The left column is the response for FBP = 0.5 µM, while the right one corresponds to FBP = 10 µM. Its clear that the NADH_m concentration reverses its dynamical behavior for low and high concentrations of FBP. See the text for further details

the increasing of the relaxation times, for higher concentrations of FBP we observe a smoothing out of the dynamical response of NADH_m. In particular, its overshooting present for large variations of CA_c and low FBP disappears for the high FBP concentration case, compare Figs. 5 and 6. By examining this overshooting in the dynamics...
Fig. 5 Response of Eqs. (33)–(36) to step-like inputs (38). The magenta, blue, red, and green curves correspond, respectively, to $t_0 = 2.5, 1, 0.5,$ and $0.02$ s. The inertia of the system increases considerably for higher values of $u$, see the text for further details. The curves were evaluated for $\text{FBP} = 0.5 \, \mu\text{M}$ (Color figure online).

of NADH$_m$, our most rapid variable, it is possible to estimate the critical time for which any adiabatic approximation should break. We can see from Figs. 5 and 6 that the overshooting appears for transitions occurring in less than 2.5 s approximately. We do not expect any stationary response for input variables varying over periods smaller than this.
4 Final Remarks

We have revisited here the mathematical model for ATP production in mitochondria introduced recently by Bertram, Pedersen, Luciani, and Sherman (BPLS) in
Bertram et al. (2006) as a simplification of the more complete but intricate Magnus and Keizer’s model (Magnus and Keizer 1997, 1998a, 1998b). We checked carefully all the approximations introduced in the BPLS model and found some inaccuracies for the approximations used for the adenine nucleotide translocator rate $J_{\text{ANT}}$ and for the calcium uniporter rate $J_{\text{uni}}$. We proposed some enhanced approximations for such rates based on the original Magnus and Keizer’s model and analyzed some dynamical properties of the model. Our results for the stationary regime indicate that the BPLS model is indeed globally stable, reinforcing its relevance to physiological quantitative studies, despite its simplicity when compared to the Magnus and Keizer’s model. We have considered also the nonstationary regime and detected a effect, which could be, in principle, physiologically interesting: The inertia of the system tends to increase considerably for high concentrations of cytosolic calcium and FBP, i.e., some response times of the model tend to increase considerably for high respiration inputs $C_{\text{ac}}$ and FBP. In particular, for $C_{\text{ac}} \approx 0.2 \mu M$ and FBP $\approx 0.5 \mu M$, approximately 10 s are necessary to NADH$_m$ and $\Delta V$ attain homeostasis after a sudden increasing in $C_{\text{ac}}$. The variables ATP$_m$ and Ca$_m$ are typically slower and need approximately 30 s to attain homeostasis in the same conditions. Keeping FBP constant and increasing $C_{\text{ac}}$, or keeping $C_{\text{ac}}$ and increasing FBP, will imply a considerably increasing of this response time, i.e., the system will take a longer time to attain homeostasis.

It is interesting to notice that the dynamics of our enhanced model are qualitatively similar to the original BPLS one, despite the differences in the rates $J_{\text{ANT}}$ and $J_{\text{uni}}$ for physiological ranges, as depicted, for instance, in Fig. 1. This point can be understood from the fact that the value of $w^*$, which does not depends tightly on the details of such rates, is almost constant and corresponding to $\Delta V = 150 \text{ mV}$ for reasonable values of the inputs $v^*$ and $u^*$. For a fixed value of $\Delta V$, the numerical parameters in (11) can be fitted to provide a good adjustment for the real ATP dependence of (13). An inspection of Fig. 9 of Bertram et al. (2006) reveals that the adjustment of their numerical parameters was probably checked for ATP $\approx 3 \text{ mM}$ and for $\Delta V \approx 160 \text{ mV}$, which is close to the physiological global fixed point (homeostasis), explaining why the asymptotic dynamics are not strongly affected by the inaccuracies in the BPLS approximations. On the other hand, we do not expect that the detected nonstationary effects be independent on the details of $J_{\text{ANT}}$ and $J_{\text{uni}}$. Such points certainly deserve further investigation.

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