MODEL EQUATIONS and PARAMETERS: Integrated Computational Modeling of Oxygen Transport and Cellular Energetics Explains Observations on In Vivo Cardiac Oxygen Consumption and Energy Metabolites

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Governing equations for oxygen transport

Oxygen transport in the capillary is governed by advection of the blood, oxygen-hemoglogin binding and unbinding, and passive permeation between the blood and the interstitial space. The governing equation for oxygen in the capillary is

$$\frac{\partial C_{\mathrm{O},1}}{\partial t} = -\frac{\rho G L}{V_1}\frac{\partial C_{\mathrm{O},1}}{\partial x} - \frac{\alpha_1 PS_{12}}{V_1}\left(P_1 - P_2\right) \qquad (1)$$

where, $C_{O,1}(x,t)$ is the total oxygen concentration in the blood (free plus hemoglobin-bound oxygen), G is blood flow to the tissue in volume per unit time per mass of tissue, ρ is the tissue density in mass per unit volume, L is the length of the capillary, V_1 is the volume of the capillary region, α_1 is the oxygen solubility coefficient in blood, PS_{12} is the permeability of the surface area of capillary wall, and P_1 and P_2 are the oxygen partial pressures in blood and interstitial fluid, respectively. The capillary oxygen concentration $C_{O,1}$ is a function of the distance along the capillary, x, and time, t.

The total oxygen concentration of the capillary region is related to the partial pressure by

$$C_{\mathrm{O},1} = \alpha_1 P_1 + Hct\, C_{\mathrm{Hb}}\, S_{\mathrm{Hb}}\,, \qquad (2)$$

where Hct is the hematocrit, C_{Hb} is the concentration of oxygen binding sites in red blood cells and S_{HB} is the hemoglobin saturation in red blood cells. The hemoglobin saturation curve is assumed to be governed by a Hill equation,

$$S_{\mathrm{Hb}} = \frac{P_1^{n_H}}{P_1^{n_H} + P_{50,\mathrm{Hb}}^{n_H}} \tag{3}$$

where $P_{50,\mathrm{Hb}}$ is half the partial pressure of O_2 saturation in hemoglobin and n_H is the Hill exponent.

Oxygen transport in the interstitial region is governed by passive permeation of oxygen between the blood and the interstitial fluid and between the interstitial fluid and the myocyte

$$\frac{\partial C_{\mathrm{O},2}}{\partial t} = \frac{\alpha_1 PS_{12}}{V_2}(P_1 - P_2) - \frac{\alpha_1 PS_{23}}{V_2}(P_2 - P_3) \tag{4}$$

where, $C_{\mathrm{O},2}(x,t)$ is the oxygen concentration in interstitial region, V_2 is the volume of the interstitial region, PS_{23} is the permeability surface-area product for passive permeation between interstitium region and the parenchymal cell region, and P_3 is the partial pressure of the oxygen in the cell. Oxygen concentration and partial pressure in the interstitial fluid are related by $C_{\mathrm{O},2} = \alpha_2 P_2$, where α_2 is the oxygen solubility coefficient in the interstitial region,

In the cellular region, oxygen transport is governed by passive permeation and consumption of oxygen in mitochondria

$$\frac{\partial C_{\mathrm{O},3}}{\partial t} = \frac{\alpha_1 PS_{23}}{V_3}(P_2 - P_3) - R_{\mathrm{MitoCell}}\left(\frac{J_{\mathrm{C4}}}{2}\right) \tag{5}$$

where $C_{\mathrm{O},3}$ is the oxygen concentration in the cellular region, V_3 is the volume of the cellular region, R_{MitoCell} is the ratio of mitochondrial volume to total cell volume, and J_{C4} is the flux through Complex IV in the respiratory chain in units of mass per time per unit mitochondrial volume. The factor of 1/2 in second term on the RHS of Equation (5) accounts for the fact that J_{C4} in the mitochondrial model (described below) represents the flux of electron pairs through the respiratory chain: one O_2 molecule is consumed for each two pairs of electrons transferred through the system. Equation (5) assumes that mitochondria are uniformly and homogeneously distributed in the cellular space.

Total oxygen in the cell is the sum of free oxygen plus myoglobin-bound oxygen

$$C_{\mathrm{O},3} = \alpha_3 P_3 + C_{\mathrm{Mb}} S_{\mathrm{Mb}}, \tag{6}$$

where C_{Mb} is the myoglobin concentration in the cell and α_3 is the oxygen solubility in the myocyte. The equilibrium myoglobin saturation S_{Mb} is expressed

$$S_{\mathrm{Mb}} = \frac{P_3}{P_3 + P_{50,\mathrm{Mb}}}, \tag{7}$$

where $P_{50,\mathrm{Mb}}$ is the half-saturation partial pressure for oxygen-myoglobin binding.

Governing equations for cellular energetics

The differential equations for species other than oxygen are

$$\partial[\mathrm{H}^+]_\mathrm{x}/\partial t = \beta^{-1}[\mathrm{H}^+]_\mathrm{x}\left(+J_{DH} - 5J_{C1} - 2J_{C3} - 4J_{C4} + (n_A - 1)J_{F1} + 2J_{\mathrm{PiHt}} - J_{\mathrm{KH}} + J_{\mathrm{Hle}}\right)/\, W_\mathrm{x}$$

$$\partial[\mathrm{K}^+]_\mathrm{x}/\partial t = \left(+J_{KH}\right)/\, W_\mathrm{x}$$

$$\partial[\mathrm{Mg}^{2+}]_\mathrm{x}/\partial t = \left(-J_{\mathrm{MgADPx}} - J_{\mathrm{MgATPx}}\right)/\, W_\mathrm{x}$$

$$\partial[\mathrm{NADH}]_\mathrm{x}/\partial t = \left(+J_{\mathrm{DH}} - J_{\mathrm{C1}}\right)/\, W_\mathrm{x}$$

$$\partial[\mathrm{Q}]_\mathrm{x}/\partial t = \left(-J_{\mathrm{C1}} + J_{\mathrm{C3}}\right)/\, W_\mathrm{x}$$

$$\partial[\mathrm{cytC(ox)}^{2+}]_\mathrm{i}/\partial t = \left(-2J_{\mathrm{C3}} + 2J_{\mathrm{C4}}\right)/\, W_\mathrm{i}$$

$$\partial[\mathrm{ATP}]_\mathrm{x}/\partial t = \left(+J_{\mathrm{F_1}} - J_{\mathrm{ANT}}\right)/\, W_\mathrm{x}$$

$$\partial[\mathrm{mATP}]_\mathrm{x}/\partial t = \left(+J_{\mathrm{MgATPx}}\right)/W_\mathrm{x}$$

$$\partial[\mathrm{mADP}]_\mathrm{x}/\partial t = \left(+J_{\mathrm{MgADPx}}\right)/\, W_\mathrm{x}$$

$$\partial[\mathrm{Pi}]_\mathrm{x}/\partial t = \left(-J_{\mathrm{F_1}} + J_{\mathrm{PiHt}}\right)/\, W_\mathrm{x}$$

$$\partial[\mathrm{ATP}]_\mathrm{i}/\partial t = \left(+J_{\mathrm{ATPt}} + J_{\mathrm{ANT}} + J_{\mathrm{AKi}}\right)/\, W_\mathrm{i}$$

$$\partial[\mathrm{ADP}]_\mathrm{i}/\partial t = \left(+J_{\mathrm{ADPt}} - J_{\mathrm{ANT}} - 2J_{\mathrm{AKi}}\right)/\, W_\mathrm{i}$$

$$\partial[\mathrm{AMP}]_\mathrm{i}/\partial t = \left(+J_{\mathrm{AMPt}} + J_{\mathrm{AKi}}\right)/\, W_\mathrm{i}$$

$$\partial[\mathrm{mATP}]_\mathrm{i}/\partial t = \left(+J_\mathrm{MgATPi}\right)\Big/ W_\mathrm{i}$$

$$\partial[\mathrm{mADP}]_\mathrm{i}/\partial t = \left(+J_\mathrm{MgADPi}\right)\Big/ W_\mathrm{i}$$

$$\partial[\mathrm{Pi}]_\mathrm{i}/\partial t = \left(+J_\mathrm{Pit} - J_\mathrm{PiHt}\right)\Big/ W_\mathrm{i}$$

$$\partial[\mathrm{Mg}^{2+}]_\mathrm{i}/\partial t = \left(-J_\mathrm{MgADPi} - J_\mathrm{MgATPi}\right)\Big/ W_\mathrm{i}$$

$$\partial[\mathrm{ATP}]_\mathrm{c}/\partial t = \left(-R_\mathrm{MitoCyto}J_\mathrm{ATPt} - R_\mathrm{CellCyto}J_\mathrm{AtC} + J_\mathrm{AKc} + J_\mathrm{CKc}\right)\Big/ W_\mathrm{c}$$

$$\partial[\mathrm{ADP}]_\mathrm{c}/\partial t = \left(-R_\mathrm{MitoCyto}J_\mathrm{ADPt} + R_\mathrm{CellCyto}J_\mathrm{AtC} - 2J_\mathrm{AKc} - J_\mathrm{CKc}\right)\Big/ W_\mathrm{c}$$

$$\partial[\mathrm{AMP}]_\mathrm{c}/\partial t = \left(-R_\mathrm{MitoCyto}J_\mathrm{AMPt} + J_\mathrm{AKc}\right)\Big/ W_\mathrm{c}$$

$$\partial[\mathrm{MgATP}]_\mathrm{c}/\partial t = \left(+J_\mathrm{MgATPc}\right)\Big/ W_\mathrm{c}$$

$$\partial[\mathrm{MgADP}]_\mathrm{c}/\partial t = \left(+J_\mathrm{MgADPc}\right)\Big/ W_\mathrm{c}$$

$$\partial[\mathrm{Pi}]_\mathrm{c}/\partial t = \left(-R_\mathrm{MitoCyto}J_\mathrm{Pit} + R_\mathrm{CellCyto}J_\mathrm{AtC}\right)\Big/ W_\mathrm{c}$$

$$\partial[\mathrm{Mg}^{2+}]_\mathrm{c}/\partial t = \left(-J_\mathrm{MgADPc} - J_\mathrm{MgATPc}\right)\Big/ W_\mathrm{c}$$

$$\partial[\mathrm{CrP}]_\mathrm{c}/\partial t = \left(-J_\mathrm{CKc}\right)\Big/ W_\mathrm{c}$$

$$\partial\Delta\Psi/\partial t = (+4J_\mathrm{C1} + 2J_\mathrm{C3} + 4J_\mathrm{C4} - n_\mathrm{A}J_\mathrm{F1} - J_\mathrm{ANT} - J_\mathrm{Hle})/C_\mathrm{IM}\ . \qquad (8)$$

In the above set of equations, the suscripts “x”, “i”, and “c”, denote mitochondrial matrix, intermembrane space, and cytoplasm, respectively. All of the variables in this set of equations are defined in Table 1.

In addition to the state variables treated in Equations (1), (4), (5), and (8), the concentrations of several species are computed

$$\begin{aligned}
[\mathrm{NAD}]_\mathrm{x} &= \mathrm{NAD}_\mathrm{tot} - [\mathrm{NADH}]_\mathrm{x} \\
[\mathrm{QH_2}] &= \mathrm{Q}_\mathrm{tot} - [\mathrm{Q}] \\
[\mathrm{cytC(red)}^{3+}] &= \mathrm{cytC}_\mathrm{tot} - [\mathrm{cytC(ox)}^{2+}] \\
[\mathrm{ADP}]_\mathrm{x} &= \mathrm{A}_\mathrm{tot} - [\mathrm{ATP}]_\mathrm{x} \\
[\mathrm{Cr}]_\mathrm{c} &= \mathrm{CR}_\mathrm{tot} - [\mathrm{CrP}]_\mathrm{c}
\end{aligned} \qquad (9)$$

where NAD_{tot}, Q_{tot}, $cytC_{tot}$, and A_{tot}, are the total concentrations of NAD(H), ubiquinol, cytochrome c, and adenine nucleotide in the matrix, respectively, and CR_{tot} is the total creatine plus creatine phosphate concentration in the cytoplasm.

Parameters that appear in the above equations are described in detail below. The fluxes that appear on the right-hand side of the governing equations are tabulated in Table 2. For mitochondrial species, the governing equations follow from ref. [1]. For cytoplasmic species, the reactions modeled are ATP consumption, creatine kinase reaction, adenylate kinase reaction, and transport between the cytoplasm and the mitochondrial intermembrane space.

Mathematical expressions for mitochondrial fluxes

Flux expressions for the mitochondrial model (based on a previously developed model [1]) are listed below. Definitions of the variables and parameters that appear in the following expressions are listed in Tables 2 and 3.

Dehyhdrogenase flux:

$$J_{DH} = X_{DH}\left(\frac{1+[\mathrm{Pi}]_x/k_{\mathrm{Pi},1}}{1+[\mathrm{Pi}]_x/k_{\mathrm{Pi},2}}\right)\left(r\,[\mathrm{NAD}]_x - [\mathrm{NADH}]_x\right). \tag{10}$$

Complex I flux:

$$J_{C1} = X_{C1}\left(e^{-\left(\Delta G_{o,\,C1}+4\Delta G_H - RT\ln\left([H^+]_x/10^{-7}\right)\right)/RT}[\mathrm{NADH}]_x[Q] - [\mathrm{NAD}]_x[QH_2]\right), \tag{11}$$

Where $\Delta G_H = F\Delta\Psi + RT\ln\left([H^+]_c/[H^+]_x\right)$.

Complex III flux:

$$J_{C3} = X_{C3}\left(\frac{1+[\mathrm{Pi}]_x/k_{\mathrm{Pi},3}}{1+[\mathrm{Pi}]_x/k_{\mathrm{Pi},4}}\right)\cdot\left(e^{-\left(\Delta G_{o,\,C3}+4\Delta G_H-2F\Delta\Psi\right)/2RT}[\mathrm{cytC(ox)}^{3+}][QH_2]^{1/2} - [\mathrm{cytC(red)}^{2+}][Q]^{1/2}\right). \tag{12}$$

Complex IV flux:

$$J_{C4} = X_{C4}\left(\frac{[O_2]}{[O_2]+k_{O_2}}\right)\frac{[\mathrm{cytC(red)}^{2+}]}{\mathrm{cytC}_{tot}}\left(e^{-\left(\Delta G_{o,\,C4}+2\Delta G_H-2RT\ln\left([H^+]_x/10^{-7}\right)\right)/2RT}[\mathrm{cytC(red)}^{2+}][O_2]^{1/4} - e^{+F\Delta\Psi/RT}[\mathrm{cytC(ox)}^{3+}]\right), \tag{13}$$

where $[O_2] = \alpha_3 P_3$ is the free oxygen concentration in the cell.

F_1F_0-ATPase flux:

$$J_{F_1} = X_{F_1}\left(e^{-\left(\Delta G_{o,\,\mathrm{ATP}} - n_A \Delta G_H\right)/RT} \frac{K_{\text{Mg-ADP}}}{K_{\text{Mg-ATP}}}[\mathrm{mADP}]_x[\mathrm{Pi}]_x - (1\ \mathrm{M})[\mathrm{mATP}]_x \right). \tag{14}$$

Magnesium binding fluxes:

$$\begin{aligned} J_{\mathrm{MgATPx}} &= X_{\mathrm{MgA}}\left([\mathrm{fATP}]_x[\mathrm{Mg}^{2+}]_x - K_{\text{Mg-ATP}}[\mathrm{mATP}]_x\right) \\ J_{\mathrm{MgADPx}} &= X_{\mathrm{MgA}}\left([\mathrm{fADP}]_x[\mathrm{Mg}^{2+}]_x - K_{\text{Mg-ADP}}[\mathrm{mADP}]_x\right) \\ J_{\mathrm{MgATPi}} &= X_{\mathrm{MgA}}\left([\mathrm{fATP}]_i[\mathrm{Mg}^{2+}]_i - K_{\text{Mg-ATP}}[\mathrm{mATP}]_i\right) \\ J_{\mathrm{MgADPi}} &= X_{\mathrm{MgA}}\left([\mathrm{fADP}]_i[\mathrm{Mg}^{2+}]_i - K_{\text{Mg-ADP}}[\mathrm{mADP}]_i\right) \end{aligned} \tag{15}$$

where $[\mathrm{fATP}]_x$, $[\mathrm{fADP}]_x$, $[\mathrm{fATP}]_i$, and $[\mathrm{fADP}]_i$ denote magnesium unbound ATP in the matrix, ADP in the matrix, ATP in the IM space, and ADP in the IM space, respectively.

Substrate transport fluxes:

$$\begin{aligned} J_{\mathrm{ATPt}} &= \gamma p_A\left([\mathrm{ATP}]_c - [\mathrm{ATP}]_i\right) \\ J_{\mathrm{ADPt}} &= \gamma p_A\left([\mathrm{ADP}]_c - [\mathrm{ADP}]_i\right) \\ J_{\mathrm{AMPt}} &= \gamma p_A\left([\mathrm{AMP}]_c - [\mathrm{AMP}]_i\right) \\ J_{\mathrm{Pit}} &= \gamma p_{\mathrm{Pi}}\left([\mathrm{Pi}]_c - [\mathrm{Pi}]_i\right). \end{aligned} \tag{16}$$

Adenine nucleotide translocase (ANT) flux:

$$J_{ANT} = X_{ANT}\left(\frac{[\mathrm{fADP}]_i}{[\mathrm{fADP}]_i + [\mathrm{fATP}]_i e^{-0.35F\ \Delta\Psi/RT}} - \frac{[\mathrm{fADP}]_x}{[\mathrm{fADP}]_x + [\mathrm{fATP}]_x e^{+0.65F\ \Delta\Psi/RT}} \right)\left(\frac{1}{1 + k_{\mathrm{m,ADP}}/[\mathrm{fADP}]_i} \right). \tag{17}$$

The phosphate-hydrogen co-transporter flux:

$$J_{\mathrm{PiHt}} = X_{\mathrm{PiHt}}\left(\frac{[H_2PO_4^-]_x[H^+]_x - [H_2PO_4^-]_i[H^+]_c}{[H_2PO_4^-]_i + k_{\mathrm{PiHt}}} \right), \tag{18}$$

where $[H_2PO_4^-]_i = [H^+]_i[\mathrm{Pi}]_i/\left([H^+]_i + k_{dH}\right)$ and $[H_2PO_4^-]_x = [H^+]_x[\mathrm{Pi}]_x/\left([H^+]_x + k_{dH}\right)$.

Mitochondrial adenylate kinase flux:

$$J_{\mathrm{AKi}} = X_{\mathrm{AK}}\left(K_{\mathrm{AK}}[\mathrm{ADP}]_{\mathrm{i}}[\mathrm{ADP}]_{\mathrm{i}} - [\mathrm{AMP}]_{\mathrm{i}}[\mathrm{ATP}]_{\mathrm{i}}\right). \tag{19}$$

Proton leak flux:

$$J_{\mathrm{Hle}} = X_{\mathrm{Hle}}\Delta\Psi\left(\frac{[\mathrm{H}^+]_{\mathrm{c}}\, e^{+F\,\Delta\Psi/RT} - [\mathrm{H}^+]_{\mathrm{x}}}{e^{+F\,\Delta\Psi/RT} - 1}\right). \tag{20}$$

Potassium-hydrogen ion exchange:

$$J_{\mathrm{KH}} = X_{\mathrm{KH}}\left([\mathrm{K}^+]_{\mathrm{c}}[\mathrm{H}^+]_{\mathrm{x}} - [\mathrm{K}^+]_{\mathrm{x}}[\mathrm{H}^+]_{\mathrm{c}}\right). \tag{21}$$

The above expressions for the mitochondrial model fluxes are identical to those presented in ref. [1], with the exception of the Complex I and III fluxes, which have been modified to ensure that the model remains numerically stable when $[O_2]$ and [Q] go to zero.

Mathematical expressions for cytoplasmic reaction fluxes

Four biochemical processes are modeled in the cytoplasm—the adenylate kinase reaction, the creatine kinase reaction, ATP hydrolysis, and binding of magnesium ions to ADP and ATP.

The binding of magnesium to ATP and ADP in the cytoplasm takes the same form as the binding fluxes in the mitochondria:

$$\begin{aligned} J_{\mathrm{MgATPc}} &= X_{\mathrm{MgA}}\left([\mathrm{fATP}]_{\mathrm{c}}[\mathrm{Mg}^{2+}]_{\mathrm{c}} - K_{\mathrm{Mg\text{-}ATP}}[\mathrm{mATP}]_{\mathrm{c}}\right) \\ J_{\mathrm{MgADPc}} &= X_{\mathrm{MgA}}\left([\mathrm{fADP}]_{\mathrm{c}}[\mathrm{Mg}^{2+}]_{\mathrm{c}} - K_{\mathrm{Mg\text{-}ADP}}[\mathrm{mADP}]_{\mathrm{c}}\right) \end{aligned} \tag{22}$$

where $[\mathrm{fATP}]_{\mathrm{c}}$ and $[\mathrm{fADP}]_{\mathrm{c}}$ denote magnesium unbound ATP and ADP in the cytoplasm. Similarly, the cytoplasmic adenylate kinase is analogous to the mitochondrial reaction.

$$J_{\mathrm{AKc}} = X_{\mathrm{AK}}\left(K_{\mathrm{AK}}[\mathrm{ADP}]_{\mathrm{c}}[\mathrm{ADP}]_{\mathrm{c}} - [\mathrm{AMP}]_{\mathrm{c}}[\mathrm{ATP}]_{\mathrm{c}}\right) \tag{23}$$

In Equation (23), K_{AK} is the equilibrium constant for the reaction $2\ \mathrm{ADP} \rightleftharpoons \mathrm{ATP} + \mathrm{AMP}$, and X_{AK} is the enzyme activity, which is set to a large enough value so that the reaction is effectively maintained in equilibrium.

The creatine kinase flux is modeled using the expression:

$$J_{\mathrm{CKc}} = X_{\mathrm{CK}}\left(K_{\mathrm{CK}}[\mathrm{ADP}]_{\mathrm{c}}[\mathrm{CrP}]_{\mathrm{c}}[\mathrm{H}^+]_{\mathrm{c}} - [\mathrm{ATP}]_{\mathrm{c}}[\mathrm{Cr}]_{\mathrm{c}}\right), \tag{24}$$

where the activity X_{CK} is set to a large enough value so that the equilibrium $K_{CK} = \left([ATP]_c [Cr]_c / [ADP]_c [CrP]_c [H^+]_c \right)_{eq}$ is maintained during simulations.

The flux J_{AtC} is defined as the flux through the reaction ATP → ADP + Pi. Mathematical models for the ATP consumption flux are considered in the Results section.

Parameter values

With the exception of one adjustable parameter, all parameters in the model are fixed at values justified by previous studies. The adjustable parameter is the total pool of exchangeable phosphate in the cell, which is a constant denoted by *TPP*. The total exchangeable phosphate pool is computed as

$$\begin{aligned} TPP = \Big[& V_{cyto} W_c \left(2[ATP]_c + [ADP]_c + [Pi]_c + [CrP]_c \right) \\ & + V_{mito} W_i \left(2[ATP]_i + [ADP]_i + [Pi]_i \right) \\ & + V_{mito} W_x \left([ATP]_x + [Pi]_x \right) \Big] / V_{cell} \end{aligned} \tag{25}$$

where V_{cyto} = 0.472 ml (g tissue)$^{-1}$, V_{mito} = 0.200 ml (g tissue)$^{-1}$, and V_{cell} = 0.694 ml (g tissue)$^{-1}$ are the volume densities of cytoplasm, mitochondria, and total cell space in cardiac tissue [2]. By comparing simulation predictions with experimental data (see Results section), a value of *TPP* = 15 mM is chosen as the value most consistent with the experimental observations.

The parameter values listed in Table 3 are organized into oxygen transport parameters, structure/volume parameters, physicochemical parameters, mitochondrial model parameters, fixed concentration pools, and binding constants.

The oxygen transport parameters, including solubilities, permeabilities, and oxy-hemoglobin and oxy-myoglobin binding parameters are obtained from the literature, as indicated in the table. The effective permeability-surface area product for the capillary wall is obtained by converting the mass transfer coefficient reported in ref. [3] and used in ref. [4] to the corresponding *PS* product. Similarly, the structure/volume parameters are available from experimental estimations published in the literature. The effective capillary length is set to the mean capillary path length for left ventricular tissue reported by Kassab and Fung [5]. The majority of the volume density and water space measurements are obtained from the study of Vinnakota and Bassingthwaighte [2]. Values of the mitochondrial model parameters are estimated and reported in ref. [1].

Oxidative phosphorylation model parameters have been adjusted to account for modifications from ref. [1], as indicated in the table. Since the F_1F_0 ATPase reaction is maintained near chemical equilibrium in the model parameterization that was published in ref. [1], here the F_1F_0 ATPase activity X_{F1} is set to the arbitrarily high value of 1000 mol s^{-1} M^{-1} (l mito) $^{-1}$. In addition, the updates to the functional forms for the Complex I and III fluxes require estimation of values for the activities of X_{C1} and X_{C3}. To account for these changes the full set of mitochondrial model parameters has been refined by repeating the fits to data from isolated mitochondrial function of Beard [1]. The agreement between the data of Bose et al. [6] and the updated model is equivalent to that reported for the previous version of the model [1].

All values for concentrations of pooled metabolites are set according to values reported in previous studies, with the exception of the total pool of exchangeable phosphate (*TPP*), which is estimated below. Binding constants are obtained from the literature; enzyme activities for reactions maintained near equilibrium are set to arbitrarily high values.

Table 1: Model variables

Variables	Description	Units
$C_{O,1}$	Oxygen concentration in blood	mol (l blood)$^{-1}$
$C_{O,2}$	Oxygen concentration in interstitial fluid	mol (l isf) $^{-1}$
$C_{O,3}$	Oxygen concentration in myocyte	mol (l cell) $^{-1}$
P_1	Oxygen partial pressure in blood	mmHg
P_2	Oxygen partial pressure in interstitial fluid	mmHg
P_3	Oxygen partial pressure in myocyte	mmHg
S_{Hb}	Hemoglobin saturation in red blood cells	unitless
S_{Mb}	Myoglobin saturation	unitless
$[H^+]_x$	Concentration of H^+ ion in mito matrix	mol (l matrix water) $^{-1}$
$[K^+]_x$	Concentration of K^+ ion in mito matrix	mol (l matrix water) $^{-1}$
$[Mg^{2+}]_x$	Concentration of Mg^{2+} ion in mito matrix	mol (l matrix water) $^{-1}$
$[NADH]_x$	Concentration of NADH in mito matrix	mol (l matrix water) $^{-1}$
$[NAD]_x$	Concentration of NAD in mito matrix	mol (l matrix water) $^{-1}$
$[QH_2]_x$	Concentration of reduced ubiquinol in mito matrix	mol (l matrix water) $^{-1}$
$[Q]_x$	Concentration of oxidized ubiquinol in mito matrix	mol (l matrix water) $^{-1}$
$[ATP]_x$	Concentration of total ATP in mito matrix	mol (l matrix water) $^{-1}$
$[ADP]_x$	Concentration of total ADP in mito matrix	mol (l matrix water)-1
$[mATP]_x$	Concentration of Mg^{2+}-bound ATP in mito matrix	mol (l matrix water) $^{-1}$
$[mATP]_x$	Concentration of Mg^{2+}-bound ADP in mito matrix	mol (l matrix water) $^{-1}$
$[Pi]_x$	Concentration of inorganic phosphate in mito matrix	mol (l matrix water) $^{-1}$
$[cytoC(red)^{2+}]_i$	Concentration of reduced cytochrome C in IM space	mol (l IM water) $^{-1}$
$[cytoC(ox)^{2+}]_i$	Concentration of oxidized cytochrome C in IM space	mol (l IM water) $^{-1}$
$[ATP]_i$	Concentration of total ATP in IM space	mol (l IM water) $^{-1}$
$[ADP]_i$	Concentration of total ADP in IM space	mol (l IM water) $^{-1}$
$[AMP]_i$	Concentration of total AMP in IM space	mol (l IM water) $^{-1}$
$[mATP]_i$	Concentration of Mg^{2+}-bound ATP in IM space	mol (l IM water) $^{-1}$
$[mADP]_i$	Concentration of Mg^{2+}-bound ADP in IM space	mol (l IM water) $^{-1}$
$[Pi]_i$	Concentration of inorganic phosphate in IM space	mol (l matrix water) $^{-1}$
$[Mg^{2+}]_i$	Concentration of Mg^{2+} ion in IM space	mol (l IM water) $^{-1}$
$[ATP]_c$	Concentration of total ATP in myocyte	mol (l cell water) $^{-1}$
$[ADP]_c$	Concentration of total ADP in myocyte	mol (l cell water) $^{-1}$
$[AMP]_c$	Concentration of total ADP in myocyte	mol (l cell water) $^{-1}$
$[mATP]_c$	Concentration of Mg^{2+}-bound ATP in myocyte	mol (l cell water) $^{-1}$
$[mADP]_c$	Concentration of Mg^{2+}-bound ADP in myocyte	mol (l cell water) $^{-1}$
$[Mg^{2+}]_c$	Concentration of free Mg^{2+} ion in myocyte	mol (l cell water) $^{-1}$
$[Pi]_c$	Concentration of inorganic phosphate in myocyte	mol (l cell water) $^{-1}$
$[CrP]_c$	Concentration of creatine phosphate in cytoplasm	mol (l cell water) $^{-1}$
$[Cr]_c$	Concentration of creatine in cytoplasm	mol (l cell water) $^{-1}$
$\Delta\Psi$	Mitochondrial membrane potential	mV

Table 2: Model fluxes

Flux	Description	Units
Mitochondrial Matrix Reactions:		
J_{DH}	Mitochondrial dehydrogenase	mol s^{-1} (l mito)$^{-1}$
J_{C1}	Complex I	mol s^{-1} (l mito)$^{-1}$
J_{C3}	Complex III	mol s^{-1} (l mito)$^{-1}$
J_{C4}	Complex IV	mol s^{-1} (l mito)$^{-1}$
J_{F1}	F_1F_0 ATPase reaction	mol s^{-1} (l mito)$^{-1}$
J_{ANT}	Adenine nucleotide translocase	mol s^{-1} (l mito)$^{-1}$
J_{PiHt}	Phosphate-hydrogen co-transporter	mol s^{-1} (l mito)$^{-1}$
J_{Hle}	Proton leak	mol s^{-1} (l mito)$^{-1}$
J_{KH}	Mitochondrial K^+ / H^+ exchager	mol s^{-1} (l mito)$^{-1}$
J_{MgATPx}	Mg^{2+}/ATP binding in matrix	mol s^{-1} (l mito)$^{-1}$
J_{MgADPx}	Mg^{2+}/ADP binding in matrix	mol s^{-1} (l mito)$^{-1}$
Mitochondrial IM Space Reactions:		
J_{AKi}	Adenylate kinase flux in IM space	mol s^{-1} (l mito)$^{-1}$
J_{MgATPi}	Mg^{2+}/ATP binding in IM space	mol s^{-1} (l mito)$^{-1}$
J_{MgADPi}	Mg^{2+}/ADP binding in IM space	mol s^{-1} (l mito)$^{-1}$
Mitochondrial Transport Fluxes:		
J_{Pit}	Phosphate transport across outer membrane	mol s^{-1} (l mito)$^{-1}$
J_{ATPt}	ATP transport across outer membrane	mol s^{-1} (l mito)$^{-1}$
J_{ADPt}	ADP transport across outer membrane	mol s^{-1} (l mito)$^{-1}$
J_{AMPt}	AMP transport across outer membrane	mol s^{-1} (l mito)$^{-1}$
Cytoplasmic Reactions:		
J_{AKc}	Adenylate kinase flux in cytoplasm	mol s^{-1} (l cytoplasm)$^{-1}$
J_{CKc}	Creatine kinase flux in cytoplasm	mol s^{-1} (l cytoplasm)$^{-1}$
J_{MgATPc}	Mg^{2+}/ATP binding in cytoplasm	mol s^{-1} (l cytoplasm)$^{-1}$
J_{MgADPc}	Mg^{2+}/ADP binding in cytoplasm	mol s^{-1} (l cytoplasm)$^{-1}$
J_{AtC}	ATP consumption in cytoplasm	mol s^{-1} (l cell)$^{-1}$

Table 3: Parameter values

Parameter	Description	Value	Units	Reference
Oxygen Transport Parameters:				
α_1	Plasma O_2 solubility	1.30×10^{-6}	M $mmHg^{-1}$	[7]
α_2	Interstitial fluid O_2 solubility	1.25×10^{-6}	M $mmHg^{-1}$	[8]
α_3	Myocyte O_2 solubility	1.74×10^{-6}	M $mmHg^{-1}$	[9]
PS_{12}	Capillary wall PS product	50	ml s^{-1} (ml tissue) $^{-1}$	[3, 4]
PS_{23}	Myocyte fiber PS product	10	ml s^{-1} (ml tissue) $^{-1}$	[10]
Hct	Hematocrit	0.45	unitless	
C_{Hb}	Oxy-hemoglobin binding site concentation	0.0213	mol $(l\ rbc)^{-1}$	[7]
$P_{50,Hb}$	Hemoglobin half-saturation pO_2	30.0	mmHg	[11]
n_H	Hemoglobin Hill coefficient	2.55	unitless	[11]
C_{Mb}	Myoglobin concentration	200×10^{-6}	mol (l cell) $^{-1}$	
$P_{50,Mb}$	Myoglobin half-saturation pO_2	2.39	mmHg	[12]
P_{input}	Arterial oxygen pO_2	100	mmHg	
Structure/Volume Parameters:				
ρ	Tissue density	1.053	g (ml tissue) $^{-1}$	[2]
L	Capillary length	550	μm	[5]
V_1	Capillary blood volume	0.05	ml (ml tissue) $^{-1}$	[13, 14][a]
V_2	Interstitial volume	0.17585	ml (ml tissue) $^{-1}$	[2]
V_3	Cell volume	0.73078	ml (ml tissue) $^{-1}$	[2]
W_x	Matrix water space fraction	0.6514	l water (l mito) $^{-1}$	[1, 2]
W_i	IM space water fraction	0.0724	l water (l mito) $^{-1}$	[1, 2]
W_c	Cytoplasm water fraction	0.8425	l water (l cell) $^{-1}$	[2]
$R_{MitoCell}$	Mitochondrial/cell volume ratio	0.2882	l mito (l cell) $^{-1}$	[2]
$R_{MitoCyto}$	Mitochondrial/cytoplasm volume ratio	0.4237	l mito (l cell) $^{-1}$	[2]
$R_{CellCyto}$	Cell/cytoplasm volume ratio	1.4703	l cell $(l\ cytoplasm)^{-1}$	[2]
γ	Outer membrane area per mito volume	5.99	μm^{-1}	[15]
Physicochemical Parameters:				
RT	Gas constant times temperature	2.5775	kJ mol^{-1}	–[b]
F	Faraday's constant	0.096484	kJ mol^{-1} mV^{-1}	–[b]
$\Delta G_{o,C1}$	Standard free energy, Complex I	–69.37	kJ mol^{-1}	[16][c]
$\Delta G_{o,C3}$	Standard free energy, Complex III	–32.53	kJ mol^{-1}	[16][c]
$\Delta G_{o,C4}$	Standard free energy, Complex IV	–122.94	kJ mol^{-1}	[16][c]
$\Delta G_{o,ATP}$	Standard free energy, ATPase	36.03	kJ mol^{-1}	[16][c]
Mitochondrial Model Parameters:				
r	Dehydrogenase model parameter	4.559	unitless	[1, 6][d]
$k_{Pi,1}$	Dehydrogenase model parameter	0.1553	mM	[1, 6][d]

$k_{Pi,2}$	Dehydrogenase model parameter	0.8222	mM	[1, 6][d]
X_{DH}	Dehydrogenase activity	0.0866	mol s^{-1} M^{-1} (l mito) $^{-1}$	[1, 6][d]
X_{C1}	Complex I activity	4.405×10^{3}	mol s^{-1} M^{-2} (l mito) $^{-1}$	[1, 6][d]
X_{C3}	Complex III activity	4.887	mol s^{-1} $M^{-3/2}$ (l mito) $^{-1}$	[1, 6][d]
X_{C4}	Complex IV activity	6.766×10^{-5}	mol s^{-1} M^{-1} (l mito) $^{-1}$	[1, 6][d]
X_{F1}	F_1F_0-ATPase activity	1000	mol s^{-1} M^{-1} (l mito) $^{-1}$	[1, 6][d]
X_{ANT}	ANT activity	8.123×10^{-3}	mol s^{-1} (l mito) $^{-1}$	[1, 6][d]
X_{PiHt}	H^+/Pi^- co-transport activity	3.850×10^{5}	mol s^{-1} M^{-1} (l mito) $^{-1}$	[1, 6][d]
k_{PiHt}	H^+/Pi^- co-transport parameter	0.2542	mM	[1, 6][d]
X_{KH}	K^+/H^+ antiporter activity	5.651×10^{7}	mol s^{-1} M^{-2} (l mito) $^{-1}$	[1, 6][d]
X_{Hle}	Proton leak activity	200.00	mol s^{-1} M^{-1} mV^{-1} (l mito) $^{-1}$	[1, 6][d]
$k_{Pi,3}$	Complex III/Pi parameter	0.3601	mM	[1, 6][d]
$k_{Pi,4}$	Complex III/Pi parameter	5.924	mM	[1, 6][d]
n_A	H^+ stoich. coef. for F_1F_0-ATPase	3	unitless	[17]
p_{Pi}	Mitochondrial membrane permeability to inorganic phosphate	327	μm sec^{-1}	[18]
p_A	Mitochondrial outer membrane permeability to nucleotides	85.0	μm sec^{-1}	[19]
$k_{m,ADP}$	ANT Michaelis-Menten constant	3.5×10^{-6}	M	[18, 20][e]
k_{O2}	Kinetic constant for Complex IV	1.2×10^{-4}	M	[18, 20][e]
β	Matrix buffering capacity	0.01	M	[18, 20][e]
C_{IM}	Capacitance of inner membrane	6.75×10^{-6}	mol (l mito) $^{-1}$ mV^{-1}	[1, 21]
Fixed Concentrations and Concentration Pools:				
NAD_{tot}	Total matrix NAD(H) concentration	2.97	mol (l matrix water) $^{-1}$	[18, 20][e]
Q_{tot}	Total matrix ubiquinol concentration	1.35	mol (l matrix water) $^{-1}$	[18, 20][e]
$cytC_{tot}$	Total IM cytochrome c concentration	2.70	mol (l IM water) $^{-1}$	[18, 20][e]
A_{tot}	Total matrix ATP+ADP concentration	10	mol (l matrix water) $^{-1}$	[18, 20][e]
TPP	Total phosphate pool (see text)	12-18	mmol (l cell)$^{-1}$	–
$[H^+]_c$	Cytoplasm H^+ ion concentration	$10^{-7.1}$	mol (l cytoplasm water) $^{-1}$	
$[K^+]_c$	Cytoplasm K^+ ion conentration	150	mol (l cytoplasm water) $^{-1}$	
CR_{tot}	Total Cr+CrP concentration	40.14	mol (l cytoplasm water) $^{-1}$	[22][f]
Binding Constants:				
K_{AK}	Adenylate kinase equilibrium constant	0.4331	unitless	[16][c]
K_{CK}	Creatine kinase equilibrium constant	1.66×10^{9}	M^{-1}	[23]
$K_{Mg\text{-}ATP}$	Mg-ATP binding constant	24×10^{-6}	M	[24]
$K_{Mg\text{-}ADP}$	Mg-ADP binding constant	347×10^{-6}	M	[24]
X_{AK}	Adenylate kinase activity	1×10^{7}	M s^{-1} M^{-2}	–
X_{CK}	Creatine kinase activity	1×10^{7}	M s^{-1} M^{-2}	–
X_{MgA}	Mg^{2+} binding activity	1×10^{7}	M s^{-1} M^{-2}	–
k_{dH}	$H_2PO_4^-$ proton dissociate constant	1.78×10^{-7}	M	[16]

[a]Value is within range of standard values used for dog heart found in cited references.
[b]Standard physicochemical constants.
[c]Computed from thermodynamic data tabulated in cited reference.
[d]Value set by fitting the data in cited references; see text for explanation.

[e]Value used is taken from previous modeling studies, not direct experimental measure.
[f]Value is computed from (27.3 mmol (l cell water)$^{-1}$)×(1.4703 l cell (l cytoplasm)$^{-1}$).

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