**Ca2+ pushes and pulls energetics to maintain ATP balance in atrial cells: computational insights**

**Supplemental material**

Our computational model integrates several specific models, each representing a compartment or phenomenon in the cell, to jointly include all the feedback and control loops that govern the phenomena focused on in this research.

1. The sub-models include: membranal ionic currents, pumps and exchangers
2. Sarcoplasmic reticulum (SR) Ca2+ handling
3. Force generation and sarcomere energy consumption
4. Mitochondrial energy metabolism
5. Mitochondrial Ca2+ dynamics

**a.** **Membranal ionic currents, pumps and exchangers**

This part of the model is based on the rabbit atrial cell model described by Lindblad et al. (1), which is presented here for the readers’ convenience. Their model consists of two components: an equivalent electrical circuit corresponding to the sarcolemmal ionic channels, pumps and exchangers and a model of the intracellular space molecules and SR compartments, presented in the next section.

The sarcolemma is modeled as a capacitor (Cm), powered by various ionic currents, pumps and exchangers, which have been previously shown to exist in the rabbit atrial cell membrane (2). We assumed that the transmembranal potential (V) is spatially uniform and time varying, and that the total net ionic current () is linearly related to the time derivative of V:

1. .

This model utilizes Hodgkin-Huxley (HH) style equations for each ionic current using the following general equation:

1. ,

where Ei is the reversal potential for the ith ionic species, is the maximal conductance for the ith current, and the terms are HH-type gating variables with the exponents for the ith ionic current characteristics.

The gating variables, through HH equation characteristics, follow first-order kinetics through the general equation:

1. ,

where represents the steady state value of y as a function of V and is the voltage-dependent time constant of y.

To summarize, this part of our model assumes that: (i) the extracellular ionic concentrations are constant, (ii) the cell model is a single-component model for the distribution of Na+ and K+, (iii) the model equations are based on HH formalism, and (iv) the atrial cell possesses a uniform cylindrical geometry.

The reversal (“Nernst”) potentials of the main ions are calculated by:

1. ,

whereand are the ion concentrations in the outside and in the cytoplasm space, respectively, R is the ideal gas constant, T is the temperature in Kelvin and F is Faraday’s constant.

The model parameters are summarized in Table S1 and the initial values of the state variables are presented in Table S2.

*i. Fast-delayed rectifier current*

2. ,

where is the maximal conductance for the fast-delayed rectifying channels.

*ii. Slow-delayed rectifier current*

1. ,

where is the maximal conductance for the slow-delayed rectifying channels.

*iii. Inward rectifier channel*

1. ,

where is the maximal conductance of the Inward rectifier channels and is the equilibrium binding constant for K+ dependence of the Inward rectifier channel channels.

*iv. Transient outward channel*

1. ,

where is the maximal conductance of the transient outward channels.

*v. Sustained outward current*

1. ,

where is the maximal conductance of the sustained outward current and is the reversal potential of the sustained outward currents.

*vi. Na+-K+- ATPase*

1. ,

where is the maximal current of the Na-K pumps, is the equilibrium binding constant of K+ to the pump and is the equilibrium binding constant of Na+.

*vii. Na+-Ca2+ exchanger (NCX)*

1. ,

where is the position of Eyring rate theory energy barrier controlling voltage dependence of the NCX, and is a empirically fitted constant .

*viii. Fast Na+ channels*

1. ,

|where is the maximal conductance of the fast sodium channels.

*ix. Membranal Ca2+ pump*

1. ,

where is the maximal current of the membranal pumps.

*x. L-type Ca2+ channels*

1. ,

where is the maximal conductance of the L-type calcium channels and is the reversal potential for the L-type calcium channels. Note that doesn’t necessary match the Nernst potential of Ca2+ in the cell.

*xi. T-type Ca2+ channels*

1. ,

where is the maximal conductance of the T-type calcium channels and is the reversal potential for the T-type calcium channels. Note that doesn’t necessarily match the Nernst potential of Ca2+ in the cell.

*xii. Background currents*

1. ,

where and are the leakage conductance for Na+ and Ca2+, respectively.

*xiii. Total membranal current*

*xiv. Sodium and potassium concentrations in cytoplasm*

1. ,

where is the intracellular volume and is the cytoplasmic volume.

**Table S1: Ionic current, exchanger and pump parameters**

|  |  |  |  |
| --- | --- | --- | --- |
| **Units** | **Value** | **Description** | **Symbol** |
| C/m | 96847 | Faraday's constant | F |
| mJ/(M·K˚) | 8314 | Gas constant | R |
| K° | 308 | Temperature | T |
| pF | 0.05 | Cell capacitance | Cm |
| mM | 2.5 | Extracellular Ca2+ | [Ca2+]o |
| mM | 140 | Extracellular Na+ | [Na+]o |
| mM | 5 | Extracellular K+ | [K+]o |
| nL | 0.0126 | Cytoplasmic volume | Vi |
| nL | 0.005884 | Ca2+ intracellular volume | VCa |
| nL | 0.0025 | Cell volume | Vc |
| nL | 0.0003969 | SR uptake compartment volume | Vup |
| nL | 0.000044 | SR release compartment volume | Vrel |
| nS | 3.5 | Maximal conductance for fast-delayed rectifying K+ channels | gK,r |
| nS | 2.5 | Maximal conductance for slow-delayed rectifying K+ channels | gK,s |
| nS | 5.08 | Maximal conductance for inward rectifying K+ channels | gK1 |
| [] | 0.59 | Equilibrium binding constant for K+ dependence of the IK1 channels | kmK1 |
| nS | 500.2 | Maximal conductance for transient outward K+ channels | gK,to |
| nS | 2.4 | Maximal conductance for sustained outward K+ channels | gsus |
| pA | 64.41 | Maximal current of the Na+-K+ pumps | INaK,max |
| [] | 1 | Equilibrium binding constant of K+ to the pump | km,k |
| [] | 36.48 | Equilibrium binding constant of Na+ to the pump | km,Na |
| [] | 0.45 | Position of Eyring rate theory energy barrier controlling voltage dependence of NCX | γNaCa |
| [] | 0.0003 | Denominator constant for INaCa | dNaCa |
| nS | 0.003 | Maximal conductance for Na+ channels | gNa |
| pA | 9.509 | Maximal current of the membranal Ca2+ pumps | ICaP,max |
| mV | 50 | Reversal potential of L-type Ca2+ channels | ECa,L |
| nS | 2.1 | Maximal conductance of L-type Ca2+ channels | gCa,L |
| mV | 38 | Reversal potential of T-type Ca2+ channels | ECa,T |
| nS | 60 | Maximal conductance of T-type Ca2+ channels | gCa,T |
| nS | 0.03 | Leakage conductance for Na | gNa,b |
| nS | 0.03 | Leakage conductance for Ca | gCa,b |

**Table S2: Initial values of state variables**

|  |  |  |  |
| --- | --- | --- | --- |
| **Symbol** | **Description** | **Value** | **Units** |
| V | Transmembrane potential | -80 | mV |
| pa | Activation gating variable for IK,r | 4.0199∙10-5 | [] |
| pi | Inactivation gating variable for IK,r | 0.8516 | [] |
| N | Activation gating variable of IK,s | 0.0283 | [] |
| R | Activation gating variable of | 9.7411∙10-6 | [] |
| s1 | Fast inactivation gating variable for | 0.9934 | [] |
| s2 | Slow inactivation gating variable for | 0.9934 | [] |
| s3 | Third inactivation gating variable for | 0.8468 | [] |
| M | Activation gating variable for INa | 0.01309 | [] |
| h1 | Fast inactivation gating variable for INa | 0.706 | [] |
| h2 | Slow inactivation gating variable for INa | 0.6149 | [] |
| dL | Activation gating variable for ICa,L | 2.8599∙10-5 | [] |
| fL | Inactivation gating variable for ICa,L | 1 | [] |
| dT | Activation gating variable for ICa,T | 8.7458∙10-5 | [] |
| fT | Inactivation gating variable for ICa,T | 0.5951 | [] |
| [Na]i | Intracellular Na+ concentration | 8.4 | mM |
| [Ca2+]up | Ca2+ concentration in uptake compartment | 0.069 | mM |
| [Ca2+]rel | Ca2+ concentration in release compartment | 0.08 | mM |
| [Ca2+]i | Ca2+ concentration in intracellular medium | 0.0000243 | mM |
| Oc | Fractional occupancy of calmodulin by Ca2+ | 0.029108 | [] |
| OTnCa | Fractional occupancy of troponin-Ca complex by Ca2+ | 0.01407 | [] |
| OTnMgCa | Fractional occupancy of troponin-Mg complex by Ca2+ | 0.214 | [] |
| OTnMgMg | Fractional occupancy of troponin-Mg complex by Mg2+ | 0.6936 | [] |
| Ocalse | Fractional occupancy of calsequestrin by Ca2+ | 0.09674 | [] |
| Ko | Extracellular K+ concentration | 5 | mM |
| Ki | Intracellular K+ concentration | 140 | mM |
|  | Relative amount of inactive precursor in SR release compartment | 0.288 | [] |
|  | Relative amount of activator in SR release compartment | 0.002262 | [] |
|  | Relative amount of inactive product in SR release compartment | 0.612697 | [] |
| SL | Sarcomere length | 1.75 | µm |
| A | The density of regulatory units with bound Ca2+ and adjacent weak cross-bridges | 0.0233 | [] |
| TT | The density of regulatory units with bound Ca2+ and adjacent strong cross-bridge | 0.0018 | [] |
| U | The density of regulatory units without bound but with adjacent strong cross-bridge | 0.0293 | [] |
| Ve | Velocity | 0 | µm/sec |
| [ATP]i | EC-coupling-linked ATP concentration | 7.972 | mM |
| [Ca2+]m | Mitochondrial free Ca2+ concentration | 0.000021 | mM |
| [ADP]m | Mitochondrial ADP concentration | 0.276 | mM |
| [NADH] | Mitochondrial NADH concentration | 5.403 | mM |
| Ψm | Inner mitochondrial membrane potential | -140.7 | mV |
| [ISOC] | Isocitrate concentration (mitochondrial) | 0.41 | mM |
| [αKG] | Α-ketoglutarate concentration (mitochondrial) | 8∙10-4 | mM |
| [SCoA] | Succinyl-CoA concentration (mitochondrial) | 0.362∙10-3 | mM |
| [­Suc] | Succinate concentration (mitochondrial) | 1.06∙10-4 | mM |
| [FUM] | Fumarate concentration (mitochondrial) | 0.0282 | mM |
| [MAL] | Malate concentration (mitochondrial) | 0.01316 | mM |
| [OAA] | Oxalacetate concentration (mitochondrial) | 2.367∙10-4 | mM |
| [FLV] | Flavoprotein concentration | 5.403 | mM |
| [AcCoA] | Acetyl CoA concentration | 1 | mM |

**b.** **Sarcoplasmic Reticulum (SR) and Ca2+ Handling**

The modeling of Ca2+ concentration in the cell is based on division of the cell into several inner compartments, each with different calcium buffering capacities. In this section, we model the buffering of all compartments and the intracellular calcium currents between compartments. The inner compartments modeled are the cytoplasm (annotated by “in”) and a SR uptake and release compartment (1, 3).

The model parameters are summarized in Table S3 and the initial values of the state variables are presented in Table S2.

*i. Uptake current of Ca2+ into the SR*

1. ,

where is the maximal calcium uptake current into the SR, is the equilibrium binding concentration on the cytosolic side, is the equilibrium binding concentration in the uptake compartment of the SR side, is a translocation constant and is the Ca2+ in the uptake compartment of the SR.

*ii. Transfer current between the uptake and release compartments of the SR*

where is the calcium concentration in the release compartment of the SR, is the volume of the uptake compartment of the SR and is a transfer time constant.

*iii. Release current of Ca2+ from the SR*

1. ,

where is the rate constant for calcium release, is the volume of the release compartment of the SR, is the rate constant for recovery of the calcium-dependent calcium release channels from their inactivated state and is the equilibrium binding constant of SR release gate for . This constant takes different values in different compartments. Here we used the values in Aslanidi et al. (3).

1. ,

where is the intracellular magnesium concentration and is the intracellular volume for . The calcium buffers are – binding to calmodulin, – binding to troponin in the sarcomeres to activate the actin-myosin activity and – binding to calsequestrin in the SR. Note that calmodulin and sarcomere activities directly affect the cytoplasmatic Ca2+ concentration via , but as an SR buffer, calsequestrin affects only the SR Ca2+ concentrations. K-1 describes the dependence of Ca2+ affinity on the number of strong cross-bridges and is the rate constant of calcium binding to low-affinity troponin sites.

**Table S3: SR and Ca2+ handling parameters**

|  |  |  |  |
| --- | --- | --- | --- |
| **Units** | **Value** | **Description** | **Symbol** |
| pA | 6860 | Maximal Ca2+ uptake current into the SR | Iup,max |
| ms | 0.01 | Time transfer constant |  |
| [] | 0.045 | Equilibrium binding Ca2+ concentration on the cytosol side | Kcy,Ca |
| [] | 5 | Equilibrium binding Ca2+ concentration in the uptake compartment of the SR side | Ksr,Ca |
| [] | 0.2 | Translocation constant | Kxcs |
| pA/mM | 25000 | Rate constant for Ca2+ release | αrel |
| 1/s | 0.815 | Rate constant for recovery of the Ca2+-dependent Ca2+ release channels from their inactivated state | krecov |
| mM | 0.0003 | Equilibrium binding constant of SR Ca2+ release gate for [Ca2+]in | KM,rel |
| 1/mM/ms | 300000 | The rate constant of calcium binding to low-afﬁnity troponin sites | Fkl |

**c.** **Force generation and sarcomere energy consumption**

In the implementation we present here, we assume isometric contraction. The effect of sarcomere length on the force generated by the atrial cell is modeled based on (4).

The model parameters are summarized in Table S4 and the initial values of the state variables are presented in Table S2.

1. ,

where is the minimal length of the sarcomere, is the reciprocal of the cross-section of the tissue, is the cross-bridge-independent coefficient of Ca2+ affinity, is the cooperativity coefficient, FN is the Hill coefficient, is the half-maximal cross-bridge Ca2+ affinity, is the cross-bridge turnover rate from the weak to the strong conformation, is the cross-bridge weakening rate at isometric regime, is the mechanical feedback coefficient (describes the dependence of the XB weakening rate on the shortening velocity),  and  are constants representing the dependence of force generation on the energy state of the cell and is the unitary force per cross-bridge at the isometric regime.

1. ,

where is the maximal ATP consumption by the sarcomeres.

**Table S4: Force generation and energy consumption parameters**

|  |  |  |  |
| --- | --- | --- | --- |
| **Units** | **Value** | **Description** | **Symbol** |
| µm | 0.8 | A constant coefﬁcient that describes the effect of the actin and myosin ﬁlament lengths on the single overlap length. | SL0 |
| 1/mm2 | 2∙1013 | The reciprocal of atrial myofiber cross-section area | NC |
| 1/mM | 350 | The cross-bridge-independent coefﬁcient of calcium afﬁnity. | Fk0 |
| 1/mM | 3000 | The cooperativity coefﬁcient. Describes the dependence of calcium afﬁnity on the number of strong cross-bridges | Fk1 |
| [] | 3.5 | Hill coefficient | FN |
| 1/mm3 | 2.5·109 | Half-maximal cross-bridge Ca2+ affinity | Fk,0.5 |
| 1/ms | 40 | The cross-bridge turnover rate from the weak to the strong conformation | Ff |
| 1/ms | 30 | The cross-bridge weakening rate at isometric regime | Fg0 |
| 1/m | 4.4·106 | The mechanical feedback coefﬁcient. Describes the dependence of the XB weakening rate on the shortening velocity | Fg1 |
| mN | 2·10-9 | The unitary force per cross-bridge at isometric regime | FXB |
| mM | 0.0012 | Maximal ATP consumption by the sarcomeres | ATPmax |
| [] | 0.03 | Coefficient of force generation at the energy state of the cell |  |
| [] | 0.26 | Coefficient of force generation at the energy state of the cell |  |

**d.** **Mitochondrial energy metabolism**

This part of the model describes atrial mitochondrial metabolism, mitochondrial Ca2+ dynamics and oxygen consumption. It includes the tricarboxylic acid (TCA) cycle and its regulating enzymes, oxidative phosphorylation and the interplay between them. The calculated energy demand is the consumption of the main dynamic processes that are mainly influenced by action potentials. Other energy-demanding processes which are governed by other stimuli, such as neuronal or hormonal activations, are not modeled.

The model parameters of the TCA cycle are summarized in Table S5, oxidative phosphorylation in Table S6, and the initial values of the state variables in Table S2. As the models used in this section are based on rat ventricular cell data, model parameters were adjusted to fit data from rabbit atria based on measurements with 1 Hz stimulation frequency and then the model was tested against data from cells with 3 Hz stimulation frequency.

*i. Mitochondrial energetics and excitation contraction coupling*

Excitation contraction coupling and mitochondrial energetics are linked through ATP, adenosine diphosphate (ADP), creatine, creatine phosphate and mitochondrial and cytoplasmic Ca2+ concentrations. ATPi (EC coupling linked) is the internal ATP pool within the cytosplasm. The total concentration of adenine nucleotides is constant (CA) is the same. Therefore, the following equation applies:

1. .

*ii. The TCA cycle*

The TCA cycle was modeled as a series of reactions incorporating regulatory feedback loops similar to (5). It consists of eight steps catalyzed by eight different enzymes. This cycle harnesses the available chemical energy of acetyl CoA into the reducing power of NADH. The following equations model the main control of the TCA cycle by cellular energy demands, as represented by mitochondrial Ca2+ concentration changes and the link between glycolysis and the TCA cycle. The link to glycolysis is represented by the Ca2+-controlled pyruvate dehydrogenase activity, which serves as the source of acetyl CoA for the cycle.

We modified this energetic equation in (5) to include regulation by mitochondrial Ca2+ ([Ca2+]m) similar to (6).

1. ,

where is the is the catalytic constant of pyruvate dehydrogenase (PDH), is the pyruvate concentration and is the Michaelis constant representing the Ca2+ regulatory effect on PDH.

1. ,

where [AcCoA] is acetyl CoA concentration, OAA is oxaloacetate concentration, is the catalytic constant of citrate synthase (CS), is CS concentration, is the Michaelis constant of AcCoA, and KMOAA is the Michaelis constant of OAA.

1. .
2. ,

where is the forward rate constant of aconitase (ACO), is the equilibrium constant of ACO, [CIT] is the concentration of citrate and [ISOC] is the concentration of isocitrate.

1. ,

where is the activation constant by ADP, is the activation constant for Ca2+, is the inhibition constant by NADH, is the isocitrate dehydrogenase (IDH) rate constant, is IDH concentration, is the Michaelis constant for isocitrate, is the Michaelis constant for NAD+, is the isocitrate cooperativity, is matrix proton concentration, and and are the ionization constants of IDH.

1. .
2. ,

where is the activation constant for Mg2+, is the activation constant for Ca2+, [] is the alpha-ketoglutarate concentration, is the alpha-ketoglutarate dehydrogenase (KGDH) concentration, is the KGDH rate constant, is the Michaelis constant for NAD+, is the Michaelis constant for αKG, is the Hill coefficient of KGDH for αKG and is the Mg2+ concentration in the mitochondria.

1. .
2. ,

where is the forward rate constant of SCL, is the succinyl CoA concentration, [Suc] is the succinyl concentration and is the SCoA reaction equilibrium constant.

1. ,

where is the SDH rate constant, is the SDH enzyme concentration, is the succinate Michaelis constant, is the fumarate (FUM) inhibition constant, and is the oxaloacetate (OAA) inhibition constant.

1. ,

where is the FH forward rate constant, if the FH equilibrium constant, [FUM] is fumarate concentration and [MAL] is malate concentration.

1. ,

where , , and are MAL dehydrogenase (MDH) ionization constants, is a pH independent term in the pH activation factor of MDH, is the MDH rate constant, is the total MDH enzyme concentration, is the malate Michaelis constant, is the OAA inhibition constant, is the NAD+ Michaelis constant, [H+] is the proton matrix concentration, and [NAD] is the  nicotinamide adenine dinucleotide concentration

In summary, as we assumed that the TCA cycle is closed from the point of view of carbon intermediates, a conservation equation relating all TCA metabolites is shown here. Thus, the level of CIT is the result of the balance of all other intermediates in the cycle as follows:

is the sum of the concentration of the TCA cycle intermediate.

**Table S5: TCA cycle parameters**

|  |  |  |  |
| --- | --- | --- | --- |
| 1/ms | 0.4857 | Catalytic constant of CS | kcatCS |
| mM | 0.4 | CS concentration | ET CS |
| mM | 1.26 | AcCoA Michaelis constant | KMAcCoA |
| mM | 6.4·10-4 | OAA Michaelis constant | KMOAA |
| mM | 1 | Sum of TCA cycle intermediate concentration | CKint |
| 1/ms | 0.2587 | ACO forward rate constant | kfACO |
| [] | 0.9 | ACO equilibrium constant | KEACO |
| [] | 486.99 | ACO coefficient | KACO |
| mM | 0.62 | ADP activation constant | Ka ADP |
| mM | 0.0005 | Ca2+ activation constant | Ka Ca |
| mM | 0.19 | NADH inhibition constant | Ki,NADH |
| 1/ms | 15.3673 | IDH rate constant | kIDH cat |
| mM | 0.109 | IDH concentration | ET IDH |
| [] | 512.2446 | IDH coefficient | KIDH |
| mM | 2.5·10-5 | Matrix proton concentration | [H+] |
| mM | 8.1·10-5 | Ionization constant of IDH | kh,1 |
| mM | 5.98·10-5 | Ionization constant of IDH | kh,2 |
| mM | 1.52 | Isocitrate Michaelis constant | KM ISOC |
| [] | 2 | Isocitrate cooperativity | Ni |
| mM | 0.923 | NAD+ Michaelis constant | KM NAD |
| mM | 0.0308 | Mg2+ Activation constant | KD Mg2+ |
| mM | 6.35·10-5 | Ca2+ Activation constant | KD Ca2+ |
| mM | 0.5 | KGDH concentration | ET KGDH |
| 1/ms | 0.254828 | KGDH rate constant | kcat KGDH |
| mM | 1.94 | αKG Michaelis constant | KM αKG |
| mM | 38.7 | NAD Michaelis constant | KM NAD\_new |
| [] | 0.48 | Hill coefficient of KGDH for αKG | nαKG |
| mM | 0.4 | Mg2+ mitochondrial concentration | [Mg2+]m |
| 1/mM·ms | 238.7 | SL forward rate constant | kfSCL |
| [] | 3.115 | SL reaction equilibrium constant | KESCL |
| mM | 2·10-4 | Coenzyme A concentration | CCoA |
| 1/ms | 13.854 | SDH rate constant | kcat SDH |
| mM | 0.5 | SDH enzyme concentration | ET SDH |
| mM | 0.03 | Succinate Michaelis constant | KMSuc |
| mM | 1.3 | Fumarate inhibition constant | KiFUM |
| mM | 0.15 | Oxaloacetate inhibition constant | Ki,sdhOAA |
| 1/ms | 1.5856 | FH forward rate constant | kf FH |
| [] | 1 | FH equilibrium constant | KEFH |
| mM | 1.13·10-5 | MDH ionization constant | kh1 |
| mM | 26.7 | MDH ionization constant | kh2 |
| mM | 6.68·10-9 | MDH ionization constant | kh3 |
| mM | 5.62·10-6 | MDH ionization constant | kh4 |
| [] | 55.209 | pH-independent term in the pH activation factor of MDH | koffset |
| 1/ms | 55.7115 | MDH rate constant | kcat MDH |
| mM | 0.154 | Total MDH enzyme concentration | ET MDH |
| mM | 1.493 | Malate Michaelis constant | KMMAL |
| mM | 3.1·10-3 | Oxaloacetate inhibition constant | KiOAA |
| mM | 0.2244 | NAD+ Michaelis constant | KMNAD\_x |
| mM | 0.2∙10-4 | Ca2+-Acetyl CoA Michaelis constant | kCa,AcCoA |
| mM/s | 0.3298125 | Pyruvate dehydrogenase rate constant | kPDH |
| mM | 0.12 | Pyruvate concentration | CPYR |
| mM | 1.5 | Total sum of mitochondrial adenine nucleotides | CA |

*iii The respiration-driven proton pump*

The model is based on (5) with modification as indicated below:

1. ,

where , ,, and are the sum of products of rate constants, is the concentration of electron carriers of respiratory complexes I-III-IV, is the phase boundary potential, is the voltage correction factor, and ΔpH is the pH gradient across the inner membrane.

Mitochondrial NAD+ is assumed to be conserved according to the following relation:

1. ,

where is the total sum of mitochondrial pyridine nucleotides.

where is the equilibrium constant of respiration, is reduced FAD concentration, is oxidized FAD concentration and TotFLV is the total FADH2 and FAD concentrations.

As the reducing reagents, FADH2 and NADH are created in the TCA cycle in 1:4 ratio (FADH2:NADH) and we assume that their consumption by the electron transfer chain follows the same ratio. This means that 1/5 of oxygen consumption is attributed to FADH2-fueled and 4/5 to NADH-fueled electron transfer cycles:

1. .

In a modification of the original formulation in (5), it is also considered that the complex II electrons, input by SUC through FADH2 to the respiratory chain:

1. .

The flux of protons driven by FADH2 oxidation (VHe(F)) has the same form as VHe, except for the adjustment of the redox potential and the H+ stoichiometry. is the concentration of electron carriers (respiratory complexes II-III-IV) and is the equilibrium constant of FADH2 oxidation.

The regulation of oxidative phosphorylation by the cellular energy demands is modeled using a push and pull mechanism. ADP activates enzymes in the Krebs cycle and thereby “pushes” the respiratory flux toward ATP generation (6). In parallel, ADP controls ATP synthase by its availability, and thereby “pulls” ATP production by increasing respiratory flux (7):

1. ,

where and are the sum of products of rate constants and kATPase is defined as the coupling coefficient representing the pull effect of the ATP synthase on the activity of the electron transfer chain.

*iv. F1F0-ATPase*

According to the concept of respiratory control, mitochondrial function is governed by the availability of ADP and Pi. The chemiosmotic hypothesis dictates that ΔΨm is lowered by an H+ influx, which drives the production of ATP by F1Fo-ATPase.

1. ,

where , , ,, , and are the sum of products of rate constants, is the F1F0-ATPase concentration, is the equilibrium constant of ATP hydrolysis, is the inorganic phosphate concentration and is the total sum of mitochondrial adenine nucleotides.

*v. Adenine nucleotide translocator (ANT) and proton leak*

To complete the description of the major membrane oxidative phosphorylation-associated processes, the exchange of adenine nucleotides across the mitochondrial membrane as well as the proton leak are considered, based on (5). The ANT equation is modeled according to a sequential mechanism of the carrier. VANT, the flux of ANT-mediated exchange between cytosolic ADP and matrix ATP, is considered to be electrogenic and dependent on the gradients of both ATP and ADP across the inner mitochondrial membrane as follows:

1. ,

where is the maximal ANT rate and if the fraction of .

The proton leak is considered to be a linear function of the ΔµH through a proportionally constant given by the H+ conductance gh:

1. ,

where is the ionic conductance of the inner membrane.

**Table S6: Oxidative phosphorylation parameters**

|  |  |  |  |
| --- | --- | --- | --- |
| 1/ms | 6.394·10-13 | Sum of products of rate constants | ra |
| 1/ms | 1.76·10-16 | Sum of products of rate constants | rb |
| 1/ms | 2.656·10-17 | Sum of products of rate constants | rc1 |
| 1/ms | 8.632·10-30 | Sum of products of rate constants | rc2 |
| [] | 3.65475∙10-14 | Sum of products of rate constants | r1 |
| [] | 1.728·10-9 | Sum of products of rate constants | r2 |
| [] | 1.059·10-26 | Sum of products of rate constants | r3 |
| mM | 15.7894 | Electron carrier concentration (respiratory complexes I-III-IV) | ρres |
| [] | 1.35·1018 | Equilibrium constant of respiration | Kres |
| mM | 3.75·10-4 | Electron carrier concentration (respiratory complexes II-III-IV) | ρresF |
| mV | 1500 | Phase boundary potential | ΨB |
| [] | 0.85 | Voltage correction factor | G |
| [] | 5.765·1013 | Equilibrium constant of FADH2 oxidation | KresF |
| mM | 1.26 | Total FADH and FADH2 concentration | TotFLV |
| 1/ms | 1.656·10-8 | Sum of products of rate constants | pa |
| 1/ms | 3.337·10-10 | Sum of products of rate constants | pb |
| 1/ms | 9.651·10-7 | Sum of products of rate constants | pc1 |
| 1/ms | 7.739·10-7 | Sum of products of rate constants | pc2 |
| [] | 1.346·10-8 | Sum of products of rate constants | p1 |
| [] | 7.739·10-7 | Sum of products of rate constants | p2 |
| [] | 6.65·10-7 | Sum of products of rate constants | p3 |
| mM | 1.5·10-3 | F1-F0 ATPase concentration | ρF1 |
| [] | 1.71·106 | ATP hydrolysis equilibrium constant | KF1 |
| mM | 2 | Inorganic phosphate concentration | Pi |
| mM/ms | 1.44918411·10-5 | ANT maximal rate | VANT,max |
| [] | 0.05 | Fraction of ΔΨm | hANT |
| mM/(ms·mV) | 10-8 | Inner membrane ionic conductance | gH |
| [] | -0.6 | pH gradient across the inner membrane | ΔpH |
| mM | 10 | Total sum of mitochondrial pyridine nucleotides | CPN |

**e.** **Mitochondrial Ca2+ dynamics**

This model assumes that mitochondrial Ca2+ dynamics is controlled by three processes (8): Ca2+  influx through the Ca2+ uniporter, Ca2+ efflux through the NCX and Ca2+ buffering.

The model parameters of mitochondrial Ca2+ dynamics are summarized in Table S7 and the initial values of the state variables are sumarized in Table S2.

*i. Ca2+ uniporter*

The mitochondrial Ca2+ uniporter depends on the electrochemical driving force of Ca2+. Thus, Ψm and extracellular Ca2+ concentration are the primary determinants of the uniporter flux. The uniporter is assumed to be an ion channel permeable only to Ca2+.

The uniporter flux (Juni) can be described by:

1. ,

where is Ca2+ valence, is the Ca2+ uniporter permeability, is mitochondrial Ca2+ activity coefficient and is the extra-mitochondrial Ca2+ activity coefficient.

*ii. Na+/Ca2+ exchanger*

The NCX flux (JNC) can be described by:

1. ,

where is the Na+/Ca2+ exchanger maximal velocity, is the Na+/Ca2+ exchanger Ca2+ affinity and is the Na+/Ca2+ exchanger Na+ affinity.

**Table S7: Mitochondrial Ca2+ parameters**

|  |  |  |  |
| --- | --- | --- | --- |
| 1/ms | 2.159 | Uniporter Ca2+ permeability | PCa |
| [] | 2 | Ca2+ valence | ZCa |
| [] | 0.2 | Mitochondrial Ca2+ activity coefficient | mα |
| [] | 0.341 | Extramitochondrial Ca2+ activity coefficient | αe |
| mM/ms | 1.863·10-2 | Na+/Ca2+ exchanger maximal velocity | VNC |
| mM | 5 | Extramitochondrial Na+ concentration | Nae |
| mM | 3.96 | Mitochondrial Na+ concentration | Nae |
| [] | 1 | Ca2+ fraction that binds to Ca2+ buffers in the mitochondria | βCa |
| mM | 1.27·10-3 | KGDHC Ca2+ binding constant | KDCa |
| mM | 0.0308 | KGDHC Mg2+ binding constant | KDMg |
| mM/mV | 1.812·10-3 | Inner membrane capacitance | Cmito |

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