Supplemental Material

S1. The simplified model

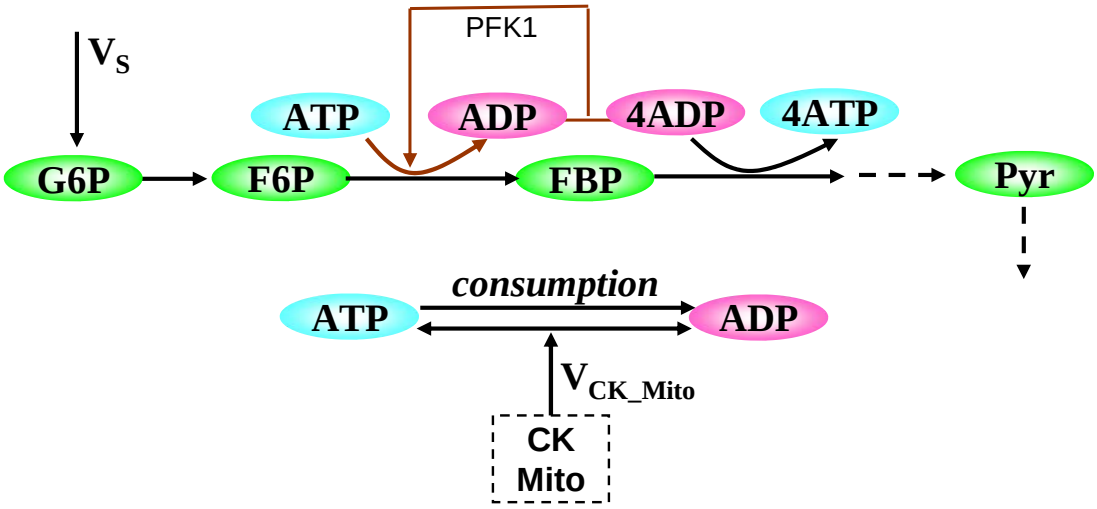

Figure S1. A schematic diagram of the simplified model of glycolysis. (Dashed arrows not formulated in equations).

Figure S1 shows a schematic plot of the simplified model of glycolysis. In this model, we assume that glucose-6-phosphate (G6P) is synthesized at the rate V_S; G6P is converted to Fructose 6-phosphate (F6P), and F6P is converted to Fructose 1,6-bisphosphate (FBP), accompanied by ATP hydrolysis to ADP; and FBP is utilized to generate Pyruvate (Pyr), accompanied by phosphorylation of four ADP to four ATP (reflecting the stochiometry for glycogenolysis). We assume that most ADP is bound, with 2.5% free ADP (ADP_F).

Here we assume that the cytoplasmic ATP consumption rate is due to various cellular ATPases: k_C*ATP, where k_C is the rate constant. The rate is assumed to be proportional to ATP concentration because oscillations occur at low ATP.

V_{CK_Mito} represents the flux of ATP from mitochondrial production (for polarized mitochondria) or consumption (for depolarized mitochondria), whose transport to the cytoplasm is facilitated by the creatine kinase (CK) shuttle. When polarized mitochondria are producing ATP, the flux of ATP from mitochondria is generated by metabolism of fatty acids and pyruvate, and is independent of intracellular ATP. However, when mitochondria are consuming ATP, V_{CK_Mito} is proportional to intracellular ATP. Thus we formulated V_{CK_Mito} as

$$V_{CK\_Mito} = \begin{cases} k_{CK} k_{Mito} c & k_{Mito} >= 0 \\ k_{CK} k_{Mito} ATP & k_{Mito} < 0 \end{cases} \quad (S1.1),$$

where k_{Mito} is the rate constant of mitochondrial ATP production or consumption and k_{CK} represents the efficiency of CK . c=1μM is a fixed constant concentration which makes the units of k_{CK} and k_{Mito} consistent between the two formulations.

The conversion of F6P to FBP is catalyzed by PFK, which is activated by free ADP. According to the earlier studies, PFK catalyzed reactions may cause glycolytic oscillations in yeast (1,2) and pancreatic β-cells (3). In our cardiac muscle cell model, we also use this positive feedback loop (activation of PFK by free ADP) to generate oscillations. We formulate PFK activity as a sigmoidal function of free ADP:

$$f_{PFK}(ADP\_F) = 0.01 + 0.99 * \frac{e^{p*ADP\_F} - 1}{(e^{p*ADP_0} - 1) + (e^{p*ADP\_F} - 1)} \quad (S1.2),$$

where $ADP_0 = 0.11$ mM, and $p = 500\,\text{mM}^{-1}$.

We assume that the total concentration of ATP and ADP (free and bound) is constant: $TAN = ADP + ATP = 6$ mM.

The differential equations of the model are:

$$
\begin{aligned}
&\frac{dG6P}{dt} = V_s - k_{g\to f} G6P \\
&\frac{dF6P}{dt} = k_{g\to f} G6P - k_{f\to f} F6P * f_{FPK}(ADP\_F) \\
&\frac{dFBP}{dt} = k_{f\to f} F6P * f_{FPK}(ADP\_F) - k_{f\to p} FBP * ADP\_F \\
&\frac{dATP}{dt} = 4 * k_{f\to p} FBP * ADP\_F - k_{f\to f} F6P * f_{FPK}(ADP\_F) - k_c \mathrm{ATP} + V_{\mathrm{CK\_Mito}} \\
&ADP = ATP_{Total} - ATP \\
&ADP\_F = ADP * 0.025
\end{aligned}
\qquad (S1.3)
$$

.

The steady state of the above equations can be obtained by setting the left-hand side of the equations to zero:

$$
\begin{aligned}
&ATP_{steady} = \begin{cases} \dfrac{3V_s + k_{Mito} k_{CK}}{k_C}, & if \quad k_{mito} \geq 0 \\ \dfrac{3V_s}{k_C - k_{Mito} k_{CK}}, & if \quad k_{mito} < 0 \end{cases} \\
&ADP_{steady} = ATP_{Total} - ATP_{steady} \\
&ADP\_F_{steady} = ADP_{steady} * 0.025 \\
&G6P_{steady} = V_s / k_{g\to f} \\
&F6P_{steady} = V_s / (k_{f\to f} f_{PFK}(ADP_{steady})) \\
&FBP_{steady} = V_s / (k_{f\to p} ADP_{steady})
\end{aligned}
\qquad (S1.4)
$$

The stabilities of the steady state can be obtained using linear stability analysis. Limit cycle oscillations occur through a Hopf bifurcation when mitochondria are consuming ATP, but not when they are producing ATP. Note that the steady state ATP, and hence also the steady state ATP/ADP_F ratio, is solely determined by the ratio of ATP production to consumption. We find that the oscillatory regime is insensitive to these parameters (Fig.1C). Thus, the ratio of the ATP production to consumption rate is the key determinant of oscillation.

S2. The three-compartment model.

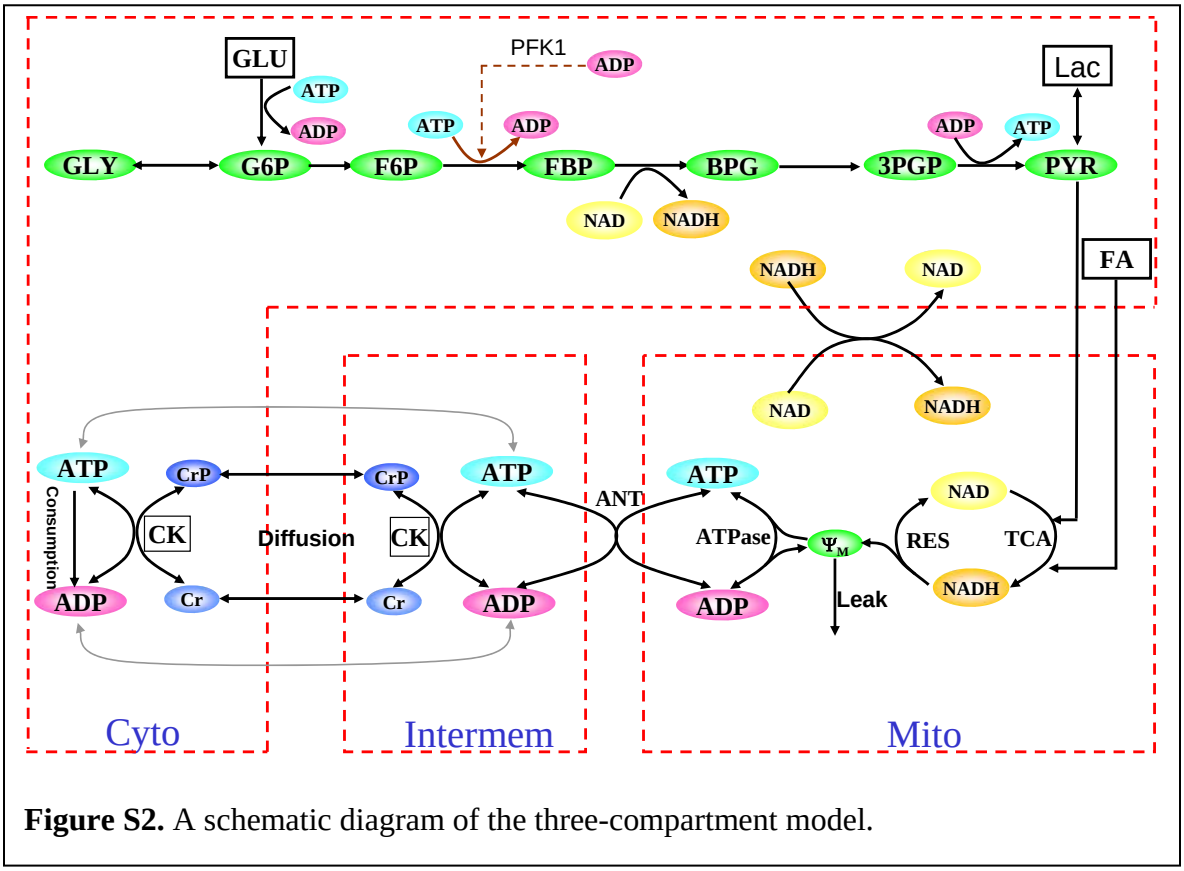

Figure S2. A schematic diagram of the three-compartment model.

Figure S2 shows a schematic plot of the detailed three-compartment model in which all variables are shown in colored ellipses, whereas parameters (assigned constant values) are shown in rectangles. The glycolytic pathways are mainly adapted from the model by Zhou et al (4). The mitochondrial metabolism model is simplified from the model by Cortassa et al (5) ATP/ADP shuttling between the glycolytic domain and the mitochondrial domain are modeled by CK diffusion (6). Following Zhou et al and Cortassa et al, some reversible reactions are modeled as forward reactions if under experimental conditions they only occur in the forward direction.

Reactions in the cytoplasm:

Based on the previous studies (4,7), we assume in our model that Glucose (Glu) and Glycogen (Gly) are utilized to generate G6P, which is sequentially converted to F6P, FBP, 1,3-Bisphosphoglycerate (BPG), 3-Phosphoglyceroyl Phosphate (3PGP), and finally Pyr. Pyruvate can either be imported into the mitochondrial matrix or converted to Lactate (Lac). Most of these reaction processes are catalyzed reactions. The conversions of Glu$\rightarrow$G6P, F6P$\rightarrow$FBP and 3-PGP $\rightarrow$Pyr are sensitive to the free ATP/ADP ratio, and the conversion of FBP$\rightarrow$BPG is sensitive to the NAD/NADH ratio.

ATP is consumed by conversion to ADP in cytoplasm. The reversible reaction of ATP with creatine (Cr) yields phosphocreatine (PCr) and ADP, and is catalyzed by CK.

Reactions in the mitochondria:

Following previous studies (4,5,8,9), we model the pathways in the mitochondrial matrix in a simplified way: Pyruvate and Fatty Acids (FA) are utilized to convert NAD to NADH via the tricarboxylic acid (TCA) cycle; O_2 consumption flux is converted by NADH (FADH) oxidation to mitochondrial membrane potential ($\Delta\Psi_m$), accompanied by proton pumping. ATP synthesis/hydrolysis through F1F0-ATPase is driven by $\Delta\Psi_m$ and mitochondrial ATP concentration.

Reactions in the inter-membrane space:

We assume that in mitochondrial intermembrane space, the reversible reaction of ATP with Cr, yielding PCr and ADP, catalyzed by CK.

Reactions shuttling through the mitochondrial membranes:

In this model, we assume that cytoplasmic Pyr, FA, and NADH are directly imported into the mitochondrial matrix (ignoring the mitochondrial inter-membrane space) and FA and Pyr are directly utilized to convert NAD to NADH. Mitochondrial free ATP/ADP are exchanged between the mitochondrial intermembrane space and the matrix through the adenine nucleotide translocator (ANT). We assume that Cr and PCr diffuse between the mitochondrial intermembrane space and the cytoplasm more rapidly than ATP and free ADP (6).

The ordinary different equations of the model:

In the differential equations and formulas, we use an abbreviation of a species to represent its concentration. For the same species located in different compartments, we use the subscripts C, I and M to denote the cytoplasm, intermembrane space and mitochondrial matrix, respectively (e.g. ATP_C, ATP_I and ATP_M). The notation $V_{x\to y}$ is used to represent the flux (reaction or transport) from *species x* to *species y*, and we assume $V_{x\to y}$ is proportional to the concentration of *species x*. For example, $V_{G6P\to F6P} = k_{G6P\to F6P}G6P$, where $k_{G6P\to F6P}$ is the rate constant. We assume that most of ADP is bound and only 2.5% of ADP is free ADP (ADP_F). In metabolic networks, many reaction processes are mediated by the phosphorylation state PS^+=*ATP/ADP_F* or redox state RS^+=*NADH/NAD*, or their opposites, PS^-= *ADP_F/ATP* or RS^-=*NAD/NADH*. In these cases, we assume $V_{x\to y}$ is also proportional to a Hill equation of the form

$\frac{PS^{\pm}}{a+PS^{\pm}}$ or $\frac{RS^{\pm}}{a+RS^{\pm}}$. For example, the reaction from FBP to BPG is driven by RS^{-}, i.e.

$V_{FBP\to G3P} = k_{FBP\to G3P} FBP \frac{RS^{-}}{a+RS^{-}}$, where $k_{FBP\to G3P}$ and a are constants.

The differential equations for the reactions in this model are as follows:

$$\frac{dGly}{dt} = -V_{Gly\to G6P}$$
$$\frac{dG6P}{dt} = V_{Gly\to G6P} + V_{Glu\to G6P} - V_{G6P\to F6P}$$
$$\frac{dF6P}{dt} = V_{G6P\to F6P} - V_{F6P\to FBP}$$
$$\frac{dFBP}{dt} = V_{F6P\to FBP} - V_{FBP\to BPG}$$
$$\frac{dBPG}{dt} = 2\cdot V_{FBP\to BPG} - V_{BPG\to 3PGP}$$
$$\frac{d3PGP}{dt} = V_{BPG\to 3PGP} - V_{3PGP\to Pyr}$$
$$\frac{dPyr}{dt} = V_{3PGP\to Pyr} - V_{Pyr\to NADH} - V_{Pyr\to Lac}$$
$$\frac{dATP_c}{dt} = -V_{ATP,consum} - V_{Glu\to G6P} - V_{F6P\to FBP} + V_{Gly\to G6P} + 2V_{3PGP\to Pyr} + V_{ATP,I\to C} / Ratio_{CI} \qquad (S2.1)$$
$$\frac{dNADH_c}{dt} = 2V_{FBP\to BPG} - V_{Pyr\to Lac} - V_{NADH\_\mathrm{Im}port}$$

$$\frac{dCr_c}{dt} = V_{PCr_C\to Cr_C} - V_{Cr,C\to I}$$

$$\frac{dPCr_c}{dt} = -V_{PCr_C\to Cr_C} + V_{PCr,I\to C} / Ratio_{CI}$$

$$\frac{dCr_I}{dt} = V_{Cr,C\to I} Ratio_{CI} - V_{Cr_I\to PCr_I}$$

$$\frac{dATP_I}{dt} = V_{ANT} / Ratio_{IM} - V_{Cr_I\to PCr_I} - V_{ATP,I\to C}$$

$$\frac{dADP_I}{dt} = -V_{ANT} / Ratio_{IM} + V_{Cr_I \to PCr_I} + V_{ADP,C \to I} Ratio_{CI}$$

$$\frac{dNADH_m}{dt} = (\frac{35}{3} + 7 * \frac{15}{3}) V_{FA \to NADH} Ratio_V + (1 + 1 * \frac{15}{3}) V_{Pyr \to NADH} Ratio_V + V_{NADH,\mathrm{Im}\ port} Ratio_V - V_{O_2}$$

$$\frac{dATP_m}{dt} = V_{ATPase} - V_{ANT}$$

$$\frac{d\Psi_m}{dt} = (V_{He} - V_{Hu} - V_{ANT} - V_{Leak}) / C_{Mito}$$

The following algebraic equations apply to the following species:

ADP_c=TAN_c-ATP_c -(ATP_I+ADP_I)/$Ratio_{CI}$

NAD_c=$Total_{NADH,c}$-$NADH_c$

ADP_m=TAN_m-ATP_m

NAD_m=$Total_{NADH,m}$

PCr_I=(Total_Cr-Cr_C-PCr_C-Cr_I/$Ratio_{CI}$)*$Ratio_{CI}$

The ratios between different volumes, the membrane capacitance C_{mito}, and the total adenine nucleotide, Cr, NADH in different compartments are listed in Table S1.

Table S1. Basic constants.

The constants are modified from the following references. Volume ratios are modified from van Beek (6); The inner membrane capacitance C_{mito} is from Cortassa et al (5); Total adenine nucleotide, Cr, NADH are modified from from Cortassa et al (5).

Description	Value
$Ratio_V = (Vol_{Cyto} + Vol_{Int}) / Vol_{Mito}$	4/1
$Ratio_{IM} = Vol_{Int} / Vol_{Mito}$	1/4

$Ratio_{CI} = Vol_{Cyto} / Vol_{In}$	15/1
Inner membrane capacitance C_{mito}	1.812 *mM/V*
Total cytoplasmic plus inter-membrane ATP and APD: TAN_c	6.4 mM
Total cytoplasmic plus inter-membrane Cr: $Total_{Cr}$	16 mM
Total cytoplasmic NADH and NAD: $Total_{NADH,c}$	0.1 mM
Total mitochondrial ATP and ADP: TAN_m	6.0 mM
Total mitochondrial NADH and NAD: $Total_{NADH,m}$	5.0 mM

The detailed description of the fluxes for the model is described below.

Flux from Glu to G6P:

$$V_{Glu\to G6P} = k_{Glu\to G6P}\frac{PS_c^+}{a+PS_c^+}Glu \qquad (S2.2)$$

where $PS_c^+ = \frac{ATP_c}{ADP\_F_c}$, $a = 5$, and $k_{Glu\to G6P} = 0.033\,s^{-1}$.

Flux from Gly to G6P:

Glycogen breakdown is catalyzed by AMP (10). Since AMP is not included in this model, we assume that glycogen breakdown is catalyzed by free ADP, which reflects AMP level:

$$f_{Pi}(ADP\_F_c) = \frac{ADP\_F_c{}^3}{0.08 + ADP\_F_c{}^3} \qquad (S2.3).$$

When glycogen concentration is low, its breakdown is turned off (11). Therefore, we use a hill equation to describe its regulation by glycogen concentration:

$$g(Gly) = \frac{Gly^5}{10^5 + Gly^5}.$$

Then the flux from Gly to G6P is:

$$V_{Gly \to G6P} = k_{\mathrm{Gly \to G6P}}\, g(Gly) * f_{Pi}(ADP\_F_c),$$

where $k_{Gly \to G6P} = 0.2\, s^{-1}$.

Flux from G6P to F6P:

$$V_{G6P \to F6P} = k_{\mathrm{G6P \to F6P}} G6P \qquad (S2.4)$$

where $k_{G6P \to F6P} = 0.075\ s^{-1}$.

Flux from F6P to FBP:

The conversion of F6P to FBP is catalyzed by PFK, which is activated by free ADP(1,2) (3).

$$V_{F6P \to FBP} = k_{\mathrm{F6P \to FBP}} F6P \frac{PS_c^+}{a + PS_c^+} f_{PFK}(ADP_c) \qquad (S2.5)$$

where $k_{F6P \to FBP} = 4.15\ s^{-1}$, $PS_c^+ = \frac{ATP_c}{ADP\_F_c}$, $a = 5$, and $f_{PFK}(ADP\_F_c)$ for which we used the following Hill equation: $f_{PFK}(ADP\_F_c) = 0.01 + 0.99 \frac{e^{500 \cdot ADP\_F_c} - 1}{e^{500 \cdot ADP\_F_c} + e^{500 \cdot .11} - 2}$.

Flux from FBP to BPG:

The flux from FBP to BPG is a multiple-step process which is simplified into one step. This process is catalyzed by RS^- (4,10):

$$V_{FBP\to BPG} = k_{FBP\to BPG}\frac{RS_c^-}{a+RS_c^-}FBP \qquad (S2.6)$$

where $RS_c^- = \frac{NAD_c}{NADH_c}$, $a = 1$, and $k_{FBP\to BPG} = 2.0\,s^{-1}$.

Flux from BPG to 3PGP:

$$V_{BPG\to 3PGP} = k_{BPG\to 3PGP}G3P \qquad (S2.7)$$

where $k_{BPG\to 3PGP} = 0.12\,s^{-1}$.

Flux from 3PGP to Pyr:

The flux from 3PGP to Pyr is a multiple-step process which is simplified into one step. This process is catalyzed by PS^- (4):

$$V_{3PGP\to 3GP} = k_{3PGP\to Pyr}\frac{PS_c^-}{a+PS_c^-}(3PGP) \qquad (S2.8)$$

where $PS_c^- = \frac{ADP\_F_c}{ATP_c}$, $a = 0.2$, and $k_{3PGP\to 3GP} = 6\,s^{-1}$.

Flux from Pyr to Lac:

The flux of Pyr to Lac is the summation of the flux from Pyr to Lac and the flux from Lac to Pyr. This process is catalyzed by RS^+ (4,10):

$$V_{Pyr\to Lac} = k_{Pyr\to Lac}\frac{RS_c^+}{a+RS_c^+}Pyr - k_{Lac\to Pyr}Lac \qquad (S2.9)$$

where $RS_c^+ = \frac{NADH_c}{NAD_c}$, $RS_c^- = \frac{NAD_c}{NADH_c}$, $a = 10$, $k_{Pyr \to Lac} = 1.3\, s^{-1}$, $k_{Lac \to Pyr} = 0.0024\, s^{-1}$.

Lac concentration is fixed at 3 mM in this model.

Flux of Pyr to NADH:

We assume that Pyr import rate is proportional to cytoplasmic Pyr concentration, and also affected by mitochondrial redox potential:

$$V_{Pyr \to NADH} = k_{Pyr \to NADH} \frac{RS_m^-}{a + RS_m^-} Pyr \qquad (S2.10)$$

where $k_{Pyr \to NADH} = 0.19\ s^{-1}$ and $a = 1$.

Flux from FA to NADH:

$$V_{FA \to NADH} = k_{FA \to NADH} \frac{RS_m^-}{a + RS_m^-} FA \qquad (S2.11)$$

where $RS_m^- = \frac{NAD_m}{NADH_m}$, $a = 1$, and $k_{Pyr \to NADH} = 0.6\, s^{-1}$.

Flux of O_2 consumption:

O_2 consumption is affected by redox potential and mitochondrial membrane potential (5,10). Here we use a simplified equation to describe it:

$$V_{O_2} = k_{O_2} \frac{RS_m^+}{a + RS_m^+} \left(1 - \frac{\Psi_m^5}{\Psi_m^5 + 0.15^5}\right) \qquad (S2.12)$$

where $RS_m^+ = \frac{NADH_m}{NAD_m}$, $a = 0.25$, and $k_{O_2} = 26\, mM/s$.

Flux of proton pumping:

Proton efflux V_{He} is linked to O_2 consumption. Here we simply assume that it is approximately proportional to O_2 consumption rate: $V_{\mathrm{He}} = 12V_{O_2}$ $(S2.13)$.

Flux of ATP synthesis/ hydrolysis:

ATP synthesis/hydrolysis through F_1/F_0-ATPase is affected by PS^- and mitochondrial membrane potential (5), i.e.,

$$V_{\mathrm{ATPase}} = k_{ATPase}(f(\Psi_M) f_{ADP}(PS_m^-) - g(\Psi_M) f_{ATP}(PS_m^+)) \quad (S2.14),$$

where $f(\Psi_M) = \frac{\Psi_M}{0.15 + \Psi_M}$, $g(\Psi_M) = 0.3*(1 - f(\Psi_M))$, $f_{ATP}(PS_m^+) = \frac{PS_m^+}{0.5 + PS_m^+}$,

$f_{ADP}(PS_m^-) = \frac{PS_m^-}{0.02 + PS_m^-}$, and $k_{ATPase} = 40\,\mathrm{mM/}s$.

Flux of proton usage by F_1/F_0-ATPase:

Proton usage V_{Hu} is linked to ATP synthesis. Here we simply assume that it is approximately proportional to ATP synthesis rate: $V_{\mathrm{Hu}} = 3V_{ATPase}$ $(S2.15)$.

Flux of ATP export/import (ANT):

ATP export/import rate through ANT is modulated by both cytoplasmic and mitochondrial ATP/ADP_F and by mitochondrial membrane potential (5), formulated as:

$$V_{\mathrm{ANT}} = k_{ANT} f(PS_{MI}) \quad (S2.16)$$

where $k_{\mathrm{ANT}} = 5\,mM/s,\ f(PS_{MI}) = \begin{cases} \dfrac{PS_{MI}}{PS_{MI}+0.5} & PS_{MI} >= 0 \\ -\dfrac{|PS_{MI}|}{|PS_{MI}|+0.5} & PS_{MI} < 0 \end{cases}$, and

$PS_{MI} = PS_m^+ * 3 - PS_I^+$.

Flux of proton leakage and usage to maintain mitochondrial functions

$$V_{\mathrm{Leak}} = k_{1,\mathrm{Leak}} e^{k_{2,Leak}(\Psi_m - 0.18)} + k_{3,Leak} \frac{\Psi_m{}^2}{\Psi_m{}^2 + 0.05} \qquad (S2.17),$$

where $k_{1,\mathrm{Leak}} = 15.0\,V/s,\ k_{2,\mathrm{Leak}} = 40\,V^{-1}$, and $k_{3,\mathrm{Leak}} = 12\ V/s$.

Flux of ATP consumption (cellular ATPases):

ATP consumption is assumed to be dependent on cytoplasmic ATP/ADP_F concentration, i.e.:

$$V_{\mathrm{ATP,consum}} = k_{\mathrm{ATP,consum}} \frac{PS_c^+}{PS_c^+ + a} \qquad (S2.18)$$

where $PS_c^+ = \dfrac{ATP_c}{ADP\_F_c}$, $a = 200$, and $k_{\mathrm{ATP,consum}} = 2.5\,mM/s$. This consumption rate is mainly determined by demand ($k_{\mathrm{ATP,consum}}$) when ATP is high, but will be determined by ATP concentration when ATP availability is low.

Flux of PCr_C to Cr_C in cytoplasm:

ATP can be synthesized from PCr_C to Cr_C (6):

$$V_{\mathrm{PCr_C}\to Cr_C} = k_{\mathrm{PCr_C}\to Cr_C}(f_{ADP}(PS_c^-) * PCr_C - f_{ATP}(PS_c^+) * Cr_C) \qquad (S2.19)$$

where $f_{ATP}(PS_c^+) = \frac{PS_c^+}{50 + PS_c^+}$, $f_{ADP}(PS_c^-) = \frac{PS_c^-}{0.004 + PS_c^-}$, $PS_c^+ = \frac{ATP_c}{ADP\_F_c}$,

$PS_C^- = \frac{ADP\_F_C}{ATP_C}$, and $k_{\mathrm{PCr_C} \to Cr_C} = 25 mM/s.$

Flux of Cr_I to PCr_I in mitochondrial inter-membrane space:

$$V_{\mathrm{Cr}_I \to PCr_I} = k_{\mathrm{Cr}_I \to PCr_I}(f_{ATP}(PS_I^+) * Cr_I - f_{ADP}(PS_I^-) * PCr_I) \quad (S2.20)$$

where $f_{ATP}(PS_I^+) = \frac{PS_I^+}{50 + PS_I^+}$, $f_{ADP}(PS_I^-) = \frac{PS_I^-}{0.004 + PS_I^-}$, $PS_c^+ = \frac{ATP_c}{ADP\_F_c}$,

$PS_C^- = \frac{ADP\_F_C}{ATP_C}$, and $k_{\mathrm{Cr}_I \to PCr_I} = 25 mM/s.$

Diffusion of PCr from mitochondrial inter-membrane space to cytoplasm:

$$V_{\mathrm{PCr,I} \to \mathrm{C}} = k_{\mathrm{PCr,I} \to \mathrm{C}}(PCr_I - PCr_C) \quad (S2.21),$$

where $k_{\mathrm{PCr,I} \to \mathrm{C}} = 150\ s^{-1}$.

Diffusion of Cr from cytoplasm to the mitochondrial intermembrane space:

$$V_{\mathrm{Cr,C} \to I} = k_{\mathrm{Cr,C} \to I}(Cr_C - Cr_I) \quad (S2.22),$$

where $k_{\mathbf{Cr,C} \to I} = 150 / Ratio_{IC} = 10\ s^{-1}$.

Diffusion of ATP from mitochondrial intermembrane space to cytoplasm:

We assume that the ATP diffusion rate through the outer mitochondrial membrane is much slower than the rates of PCr and Cr:

$$V_{\mathrm{ATP,I\to C}} = k_{\mathrm{ATP,I\to C}}(ATP_I - ATP_C) \quad (S2.23),$$

where $k_{\mathrm{ATP,I\to C}} = 0.1\ s^{-1}$.

Diffusion of Cr from cytoplasm to mitochondrial inter-membrane space:

We also assume that the free ADP diffusion rate through the outer mitochondrial membrane is much slower than the rate of diffusion of PCr and Cr:

$$V_{\mathrm{ADP,C\to I}} = k_{\mathrm{ADP,C\to I}}(ADP\_F_C - ADP\_F_I) \quad (S2.24),$$

where $k_{\mathrm{Cr,C\to I}} = 0.1/Ratio_{IC} = .00667\ s^{-1}$.

Import of NADH from cytoplasm to mitochondria:

$$V_{\mathrm{NADH,C\to M}} = k_{\mathrm{NADH,C\to M}} * f(NADH_C / NADH_M) \quad (S2.25),$$

where $k_{\mathrm{NADH,C\to M}} = 0.26\ mM/s^{-1}$ and

$$f(NADH_C / NADH_M) = (NADH_C / NADH_M)/(NADH_C / NADH_M + 0.15).$$

The reaction formulas and parameters are modified from Zhou et al (4) and Cortassa et al (5), and the diffusion rates of free ATP/ADP, Cr/PCr are adopted from Appendix B of van Beek (6). To simulate the metabolic system under normal condition, we fix $Glu = 0.5\ mM$, $FA = 0.03\ mM$, $Lac = 3.0\ mM$, and $V_{Gly\to G6P} = 0\ mM/s$. Table S2 shows the concentrations of the metabolic reactants in our model, set in a physiologically reasonable range.

Table S2. Steady state concentrations of the metabolic reactants under the normal conditions.

Glu	0.5 mM
Gly	51.7 mM
FA	0.03 mM
G6P	0.214 mM
F6P	0.398 mM
FBP	0.101 mM
BGP	0.268 mM
3PGP	0.217 mM
Pyr	0.211 mM
ATP_c	5.00 mM
Free ADP_c	0.025 mM
$NADH_c$	0.0202 mM
NAD_c	0.0798 mM
Lac	3.0 mM
Cr_c	6.14 mM
PCr_c	8.86 mM
Cr_I	6.02 mM
PCr_I	8.98 mM
ATP_I	5.01 mM
Free ADP_I	0.0188 mM
$NADH_m$	0.937 mM
NAD_m	5.01 mM
ATP_m	4.86 mM
Free ADP_m	0.0536 mM
Ψ_m	0.179 V

Simulation of the action potential duration (APD) oscillations:

To simulate the ATP oscillations causing APD oscillation, we use the ventricular action potential model by Luo and Rudy (12), as modified to include the ATP-sensitive K current formulated by Ferrero et al (12,13). The differential equation for the membrane potential V is:

$$C_m \frac{dV}{dt} = -(I_{Na} + I_{si} + I_K + I_{K1} + I_{Kp} + I_b + I_{Katp}) \qquad (S2.26),$$

where C_m = 1 μF/cm^2 is membrane capacitance, I_{Na} the fast Na current, I_{si} the L-type Ca current; I_K the time-dependent K current, I_{K1} the time-independent K current, I_{Kp} the plateau K current, I_b the background current, and I_{Katp} the ATP-sensitive K current. The formulas and parameters of all currents the same as in the original Luo and Rudy paper (12), except the formulation of I_{Katp} is described by:

$$I_{Katp} = 1.398 f_{ATP} f_m f_n (V - E_k) \qquad (S2.27),$$

where f_{ATP}, f_m, and f_n are the taken from Ferrero et al (13).

S3. Numerical simulations

The steady state stability of the simplified model (Fig 1B, 1C, 5) is solved by Matlab. The differential equations of the three-compartment model are numerically solved using programs coded in Fortran 77. We use the fourth-order Runge–Kutta method to integrate the ordinary different equations.

The parameter values used for the simulations in the figures are summarized below.

Figure 1B, 1C and 5.

$k_{G6P \rightarrow F6P}$*=0.075* s^{-1}, $k_{F6P \rightarrow FBP}$*=.625* $(mM*s)^{-1}$, $k_{FBP \rightarrow Pyr}$*=0.72* $(mM*s)^{-1}$, and k_C*=0.03* s^{-1}. k_{CK}*=0.1* and k_{CK}*=1* are used to simulate the cases with and without DNFB, respectively.

Figure 2D, 2E, 3B and 3B1.

*Glu=0 mM, FA=0.03*10% mM* (i.e. 90% blocked by 3-merc), and $k_{O_2} = 26*(0.01+0.99*e^{-0.03t})$ *mM/s* (assuming gradual block of respiration by cyanide). In the absence of exogenous glucose and fatty acids, the myocyte consumes glycogen to supply energy. To mimic the blockade of creatine kinase by DNFB, we assume $k_{Cr_I \to PCr_I} = 25*10\%$ (Eq. (S2.20)), and $k_{Cr_C \to PCr_{CI}} = 25*10\%$ (Eq. (S2.19)). All other parameters are the same as mentioned in section S2. To simulate magnesium dynamics, we assume total magnesium concentration is $Mg_{Total}=6\ mM$, and the free magnesium is $Mg_{Free}= Mg_{Total}- Mg_{Bound}$, where Mg_{Bound} equals to cytoplasmic ATP concentration.

Figure 4A1.

*Glu=0 mM, FA=0.03*10% mM*, and $k_{O_2} = 26*(0.01+0.99*e^{-0.03t})$ *mM/s*(Eq. (S2.12)). To simulate DNFB, we assume $k_{Cr_I \to PCr_I} = 25*10\%$ (Eq. (S2.20)), and $k_{Cr_C \to PCr_{CI}} = 25*10\%$ (Eq. (S2.19)). Over 400 sec, we assume that oligomycin block F_0F_1 ATPase to $k_{ATPase} = 40*2\%$ (Eq. (S2.14)).

Figure 4B1.

*Glu=0 mM, FA=0.03*10% mM*, and $k_{O_2} = 26*(0.01+0.99*e^{-0.03t})$ *mM/s* (Eq. (S2.12)). To simulate DNFB, we assume $k_{Cr_I \to PCr_I} = 25*20\%$ (Eq. (S2.20)), and $k_{Cr_C \to PCr_{CI}} = 25*20\%$ (Eq. (S2.19)). Over 400 sec, we assume that oligomycin block F_0F_1 ATPase to $k_{ATPase} = 40*5\%$ (Eq. (S2.14)).

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