

## Supplementary Information

### *Sensitivity analysis*

The sensitivity of the steady state and post-exercise concentrations in dependence of the kinetic parameters has been investigated using a global sensitivity method. Thus, the most important reactions influencing the dynamic of the system could be identified. The variations in the parameter space were generated by multiplying each parameter of the main simulation with a random factor between 1/3 and 3 using a probability distribution for the random factors, which assigned to the interval below 1 the same probability as to the interval greater than 1. We used the density-based “delta” method with a moment-independent sensitivity indicator [77,78], implemented in the python library SALIB, and generated  $10^5$  parameter variations to obtain a confidence interval of about 1 % of the sensitivity measure.

The sensitivity data in suppl. fig. 1A show that the extramitochondrial purine nucleotides (ATP, AMP, IMP) are generally most sensitive to a change in any of the model parameters due to their regulating and stabilizing effects when the ATP synthesis does not match the ATP demand. The constants for creatine kinase ( $k_{fAK}$  and  $k_{bAK}$ ) have the largest impact on the steady state concentration of ATP and by determining the ATP/ADP ratio they affect all other external species as well.

As expected the parameter  $k_{UT}$  corresponding to the ATP consumption influences the ADP, AMP, phosphate and creatine concentrations greatly, since parallel activation of ATP synthesis is not included for steady state analysis, thus creating a mismatch between ATP usage and synthesis.

The intramitochondrial species NADH and ubiquinol are mostly dependent on the first rate-limiting reaction of the mitochondrial respiration chain ( $k_{DH}$ ) and on the pyruvate availability, which in turn is determined by the ratio between forward and backward flux of lactate dehydrogenase ( $k_{fLA}$  and  $k_{bLA}$ ). The redox ratio of coenzyme c, however, is most sensitive to the  $O_2$  consumption rate at complex IV.

The sensitivity analysis of the post-exercise concentrations, shown in suppl. fig. 1B yields similar results, but with a smaller impact of creatine kinase due to the increasing influence of ATP consumption and glycolysis during high intensity exercise. Since the ATP consumption ( $k_{UT}$ ) is increased up to 300 times in exercise simulations compared to the resting state, a change of the parameter results in a relatively large mismatch of ATP usage and synthesis and strongly influences most of the species.

In line with expectations the concentration of IMP is very sensitive to the rate of ATP consumption and to the parameter for purine nucleotide degradation and loss  $k_{PL}$  during exercise, but less sensitive to all other parameters compared to the steady state analysis.

During exercise the rate of glycolysis  $k_{glyc}$  mostly influences the lactate accumulation and both the cytosolic and intramitochondrial  $H^+$  concentration. Interestingly, none of the important reactions for ATP synthesis and exchange ( $k_{SN}$ ,  $k_{glyc}$ ,  $k_{EX}$ ) has an exceptional impact on the ATP/ADP ratio in contrast to the reaction for ATP consumption, which is probably due to the complexity of regulatory and compensatory effects. If e.g. the ATP synthase was down-regulated, the intramitochondrial ATP/ADP ratio would decrease and the pH difference at the mitochondrial membrane would increase, thus leading to an upregulation of ATP synthase to a certain extent.

### *Calculations*

Total concentration of cytochrome c [30]:

$$c_t = 0.27\text{mM}, \quad (1)$$

$$c^{3+} = c_t - c^{2+}. \quad (2)$$

Total concentration of ubiquinone [30]:

$$U_t = 1.35\text{mM}, \quad (3)$$

$$UQ = U_t - UQH_2. \quad (4)$$

Total concentration of NAD [30]:

$$N_t = 2.97 \text{mM}, \quad (5)$$

$$\text{NAD}^+ = N_t - \text{NADH}. \quad (6)$$

Total creatine concentration [30]:

$$C_t = 29.5 \text{mM}, \quad (7)$$

$$\text{Cr} = C_t - \text{PCr}. \quad (8)$$

pH calculation:

$$\text{pH}_{e/i} = -\log(H_{e/i} \cdot 10^{-3}) \quad (9)$$

Extra and intramitochondrial pH buffering capacity:

$$r_{\text{buf}e} = c_{0e} \cdot \frac{d\text{pH}_e}{dH_e} = \frac{c_{0e}}{\ln 10 \cdot H_e}, \quad (10)$$

$$r_{\text{buf}i} = \frac{c_{0i}}{\ln 10 \cdot H_i}, \quad (11)$$

where  $c_{0e} = 25$  and  $c_{0i} = 22$  [30].

Potential calculation [30]:

$$\Delta \text{pH} = Z(\text{pH}_i - \text{pH}_e), \quad (12)$$

$$\Delta P = \frac{1}{1 - u} \cdot \Delta \text{pH}, \quad (13)$$

$$\Delta \Psi = -(\Delta P - \Delta \text{pH}), \quad (14)$$

$$\Delta \Psi_i = 0.65 \Delta \Psi, \quad (15)$$

$$\Delta \Psi_e = -0.35 \Delta \Psi. \quad (16)$$

Constants [30]:

$$T = 289 \text{ K}, \quad (17)$$

$$R = 0.0083 \frac{\text{kJ}}{\text{mol} \cdot \text{K}}, \quad (18)$$

$$F = 0.0965 \frac{\text{kJ}}{\text{mol} \cdot \text{mV}}, \quad (19)$$

$$Z = \ln 10 \cdot R \cdot T / F, \quad (20)$$

$$u = 0.861, \quad (21)$$

Thermodynamic span of complex I, complex III and ATP synthase [30]:

$$\Delta G_{C1} = E_{mU} - E_{mN} - 2\Delta p, \quad (22)$$

$$\Delta G_{C3} = E_{mc} - E_{mU} - (2 - u)\Delta p, \quad (23)$$

$$\gamma = 10^{\frac{\Delta G_{SN}}{Z}}, \quad (24)$$

$$\Delta G_{SN} = n_A \Delta p - \Delta G_p, \quad (25)$$

$$n_A = 2.5, \text{ (phenomenological } H^+ / \text{ATP stoichiometry of ATP synthase)} \quad (26)$$

$$\Delta G_p = 31.9 \text{ kJ/mol} / F + Z \cdot \log \left( 1000 \cdot \frac{\text{ATP}_i}{\text{ADP}_i \cdot \text{Pi}_i} \right), \quad (27)$$

$$(28)$$

Calculation of redox potentials [30]:

$$E_{mN} = -320 \text{ mV} + Z/2 \cdot \log \left( \frac{\text{NAD}}{\text{NADH}} \right), \text{ (NAD redox potential)} \quad (29)$$

$$E_{mU} = 85 \text{ mV} + Z/2 \cdot \log \left( \frac{\text{UQ}}{\text{UQH}_2} \right), \text{ (ubiquinone redox potential)} \quad (30)$$

$$E_{mc} = 250 \text{ mV} + Z \cdot \log \left( \frac{c^{3+}}{c^{2+}} \right), \text{ (cytochrome c redox potential)} \quad (31)$$

$$E_{ma} = E_{mc} + \Delta p \cdot (1 + u), \text{ (cytochrome a redox potential)} \quad (32)$$

$$A_{3/2} = 10^{\frac{E_{ma} - 540 \text{ mV}}{Z}}, \text{ (ratio } a^{3+} / a^{2+}) \quad (33)$$

$$a^{2+} = \frac{a_t}{1 + A_{3/2}}, \text{ (concentration of reduced cytochrome a)} \quad (34)$$

$$a^{3+} = a_t - a^{2+}, \quad (35)$$

$$a_t = 0.135 \text{ mM}. \quad (36)$$

In the following equations one of the subscripts refers to free (f), magnesium bound (m) or total (no subscript) concentrations and another refers to intramitochondrial (i) or extramitochondrial (no subscript) concentrations.

Calculations of free and magnesium bound ATP and ADP from [30]:

$$\text{Mg} = 4.0 \text{ mM}, \quad (37)$$

$$\text{ATP}_f = \frac{\text{ATP}}{1 + \frac{\text{Mg}}{k_{DT}}}, \quad (38)$$

$$\text{ATP}_m = \text{ATP} - \text{ATP}_f, \quad (39)$$

where  $k_{DT} = 0.024 \text{ mM}$  is the magnesium dissociation constant for external ATP.

$$\text{ADP}_f = \frac{\text{ADP}}{1 + \frac{\text{Mg}}{k_{DD}}}, \quad (40)$$

$$\text{ADP}_m = \text{ADP} - \text{ADP}_f, \quad (41)$$

where  $k_{DD} = 0.347 \text{ mM}$  is the magnesium dissociation constant for external ADP.

$$\text{Mg}_i = 0.38 \text{ mM}, \quad (42)$$

$$\text{ATP}_{fi} = \frac{\text{ATP}_i}{1 + \frac{\text{Mg}_i}{k_{DTi}}}, \quad (43)$$

$$\text{ATP}_{mi} = \text{ATP}_i - \text{ATP}_{fi}, \quad (44)$$

where  $k_{DTi} = 0.017 \text{ mM}$  is the magnesium dissociation constant for mitochondrial ATP.

$$\text{ADP}_{fi} = \frac{\text{ADP}_i}{1 + \frac{\text{Mg}_i}{k_{DDi}}}, \quad (45)$$

$$\text{ADP}_{mi} = \text{ADP}_i - \text{ADP}_{fi}, \quad (46)$$

where  $k_{DDi} = 0.282 \text{ mM}$  is the magnesium dissociation constant for mitochondrial ADP.