

Supplementary Materials for “Role of NADH/NAD⁺ Transport Activity and Glycogen Store on Skeletal Muscle Energy Metabolism during Exercise – In Silico Studies”

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APPENDIX A: DYNAMIC MASS BALANCE EQUATIONS OF O₂ AND CO₂

O₂ transport dynamics in skeletal muscle

In RBCs, O₂ is transported as free dissolved O₂ and as bound oxy-hemoglobin (HbO₂), while in mitochondria, O₂ is transported as dissolved O₂ and as oxy-myoglobin (MbO₂). In contrast, in plasma, ISF and cytosol, O₂ is transported only as dissolved O₂. Therefore, the dynamic mass balance equations of O₂ must account for different forms of O₂ transport in RBCs, plasma, ISF, cytosol and mitochondria (2; 5; 6).

The assumptions of perfect mixing in each phase and phase equilibrium between free O₂ in plasma, RBC and ISF yields $C_{bl,O_2}^F = C_{pl,O_2}^F = C_{rbc,O_2}^F = C_{isf,O_2}^F$. Furthermore, by assuming the on and off binding rates of O₂ to hemoglobin in RBCs and O₂ to myoglobin in mitochondria are fast compared to the transport processes, the detailed kinetics of the binding can be neglected and the free dissolved O₂ can be considered to be in instantaneous equilibrium with the HbO₂ in RBCs and MbO₂ in mitochondria (2).

The combined dynamic mass balance equations for O₂ in capillary blood domain in equilibrium with tissue ISF domain can be written as

$$V_{bl} \frac{dC_{bl,O_2}^T}{dt} + V_{isf} \frac{dC_{isf,O_2}^F}{dt} = Q \left(C_{art,O_2}^T - C_{bl,O_2}^T \right) - J_{bl \leftrightarrow cyt,O_2}^p \quad (A1-a)$$

and the compartmentalized dynamic mass balance equations for O₂ in tissue subcellular (cytosolic and mitochondrial) domains can be written as

$$V_{\text{cyt}} \frac{dC_{\text{cyt},O_2}^F}{dt} = J_{\text{bl} \leftrightarrow \text{cyt},O_2}^P - J_{\text{cyt} \leftrightarrow \text{mit},O_2}^P \quad (\text{A1-b})$$

$$V_{\text{mit}} \frac{dC_{\text{mit},O_2}^T}{dt} = J_{\text{cyt} \leftrightarrow \text{mit},O_2}^P - 0.5\phi_{O_2 \leftrightarrow H_2O, \text{NADH}} - 0.5\phi_{O_2 \leftrightarrow H_2O, \text{FADH}_2} \quad (\text{A1-c})$$

where the superscripts ‘F’ and ‘T’ indicate the free and total concentrations; superscript ‘p’ denotes the passive transport. In capillary blood, the concentrations are related by

$$C_{x,O_2}^T = C_{x,O_2}^F + C_{x,\text{HbO}_2}, \quad x = (\text{art}, \text{bl}) \quad (\text{A2-a})$$

and in tissue cells mitochondria, the concentrations are related by

$$C_{\text{mit},O_2}^T = C_{\text{mit},O_2}^F + C_{\text{mit},\text{MbO}_2} \quad (\text{A2-b})$$

The net transport fluxes across the cellular and mitochondrial membranes are given by

$$J_{\text{bl} \leftrightarrow \text{cyt},O_2}^P = \lambda_{\text{bl} \leftrightarrow \text{cyt},O_2} (C_{\text{bl},O_2}^F - C_{\text{cyt},O_2}^F) \quad (\text{A3-a})$$

$$J_{\text{cyt} \leftrightarrow \text{mit},O_2}^P = \lambda_{\text{cyt} \leftrightarrow \text{mit},O_2} (C_{\text{cyt},O_2}^F - C_{\text{mit},O_2}^F) \quad (\text{A3-b})$$

The concentrations of HbO₂ and MbO₂ can be written in terms of their saturations (1; 2) as

$$C_{x,\text{HbO}_2} = 4 \cdot H_{\text{rbc}} \cdot C_{\text{rbc},\text{Hb}} \cdot S_{x,\text{HbO}_2}, \quad S_{x,\text{HbO}_2} = \frac{K_{\text{HbO}_2} \cdot (C_{x,O_2}^F)^{n_H}}{1 + K_{\text{HbO}_2} \cdot (C_{x,O_2}^F)^{n_H}} \quad (\text{A4-a})$$

$$C_{\text{mit},\text{MbO}_2} = C_{\text{mit},\text{Mb}} \cdot S_{\text{mit},\text{MbO}_2}, \quad S_{\text{mit},\text{MbO}_2} = \frac{K_{\text{MbO}_2} \cdot C_{\text{mit},O_2}^F}{1 + K_{\text{MbO}_2} \cdot C_{\text{mit},O_2}^F} \quad (\text{A4-b})$$

Substituting Eq. (A4) in Eq. (A2), we have the expressions for total O₂ concentrations as

$$C_{x,O_2}^T = C_{x,O_2}^F + \frac{4 \cdot H_{\text{rbc}} \cdot C_{\text{rbc},\text{Hb}} \cdot K_{\text{HbO}_2} \cdot (C_{x,O_2}^F)^{n_H}}{1 + K_{\text{HbO}_2} \cdot (C_{x,O_2}^F)^{n_H}}, \quad x = (\text{art}, \text{bl}) \quad (\text{A5-a})$$

$$C_{\text{mit},O_2}^T = C_{\text{mit},O_2}^F + \frac{C_{\text{mit},\text{Mb}} \cdot K_{\text{MbO}_2} \cdot C_{\text{mit},O_2}^F}{1 + K_{\text{MbO}_2} \cdot C_{\text{mit},O_2}^F} \quad (\text{A5-b})$$

Substituting Eq. (A5) in Eq. (A1) and using the chain rule for differentiation and equilibrium condition $C_{\text{bl},O_2}^F = C_{\text{isf},O_2}^F$, we have the dynamic mass balance equations for O₂ as

$$(V_{\text{bl},O_2} + V_{\text{isf},O_2}) \frac{dC_{\text{bl},O_2}^F}{dt} = Q(C_{\text{art},O_2}^T - C_{\text{bl},O_2}^T) - J_{\text{bl} \leftrightarrow \text{cyt},O_2}^P \quad (\text{A6-a})$$

$$V_{\text{cyt},O_2} \frac{dC_{\text{cyt},O_2}^F}{dt} = J_{\text{bl} \leftrightarrow \text{cyt},O_2}^P - J_{\text{cyt} \leftrightarrow \text{mit},O_2}^P \quad (\text{A6-b})$$

$$V_{mit,O_2} \frac{dC_{mit,O_2}^F}{dt} = J_{cyt \leftrightarrow mit,O_2}^p - 0.5\phi_{O_2 \leftrightarrow H_2O, NADH} - 0.5\phi_{O_2 \leftrightarrow H_2O, FADH_2} \quad (A6-c)$$

where the effective volumes or volumes of distributions of O_2 in capillary blood, tissue ISF, and tissue subcellular domains (cytosol and mitochondria) (V_{bl,O_2} , V_{isf,O_2} , V_{cyt,O_2} , V_{mit,O_2}) are given by

$$V_{bl,O_2} = V_{bl} \left(1 + \frac{4 \cdot n_H \cdot H_{rbc} \cdot C_{rbc,Hb} \cdot K_{HbO_2} \cdot (C_{bl,O_2}^F)^{n_H-1}}{\left[1 + K_{HbO_2} \cdot (C_{bl,O_2}^F)^{n_H} \right]^2} \right), \quad V_{isf,O_2} = V_{isf} \quad (A7-a)$$

$$V_{cyt,O_2} = V_{cyt}, \quad V_{mit,O_2} = V_{mit} \left(1 + \frac{C_{mit,Mb} \cdot K_{MbO_2}}{\left[1 + K_{MbO_2} \cdot C_{mit,O_2}^F \right]^2} \right) \quad (A7-b)$$

CO₂ transport dynamics in skeletal muscle

In RBCs, CO₂ is transported as free dissolved CO₂, as bound carbamino-hemoglobin (HbCO₂), and as bicarbonate (HCO₃⁻), while in plasma, ISF, cytosol and mitochondria, CO₂ is transported only as dissolved CO₂ and as HCO₃⁻. Therefore, the dynamic mass balance equations for CO₂ must account for different forms of CO₂ transport in RBCs, plasma, ISF, cytosol and mitochondria (2; 4).

The assumptions of perfect mixing in each phase and phase equilibrium between free CO₂ in plasma, RBC and ISF yields $C_{bl,CO_2}^F = C_{pl,CO_2}^F = C_{rbc,CO_2}^F = C_{isf,CO_2}^F$. Furthermore, by assuming that the on and off binding rate of CO₂ to hemoglobin in RBCs is fast compared to the transport processes, the detailed kinetics of the binding can be neglected and the dissolved CO₂ can be considered to be in instantaneous equilibrium with the HbCO₂ in blood (2; 4).

The combined dynamic mass balance equation for CO₂ in capillary blood domain in equilibrium with tissue ISF domain can be written as

$$V_{bl} \frac{dC_{bl,CO_2}^T}{dt} + V_{isf} \frac{dC_{isf,CO_2}^T}{dt} = Q(C_{art,CO_2}^T - C_{bl,CO_2}^T) - J_{bl \leftrightarrow cyt,CO_2}^p \quad (A8-a)$$

and the compartmentalized dynamic mass balance equations for CO₂ in tissue subcellular (cytosolic and mitochondrial) domains can be written as

$$V_{cyt} \frac{dC_{cyt,CO_2}^T}{dt} = J_{bl \leftrightarrow cyt,CO_2}^p - J_{cyt \leftrightarrow mit,CO_2}^p \quad (A8-b)$$

$$V_{mit} \frac{dC_{mit,CO_2}^T}{dt} = J_{cyt \leftrightarrow mit,CO_2}^p + (\phi_{PYR \leftrightarrow ACoA} + \phi_{CIT \leftrightarrow AKG} + \phi_{AKG \leftrightarrow SCoA}) \quad (A8-c)$$

where

$$C_{x,CO_2}^T = C_{x,CO_2}^F + C_{x,HbCO_2} + C_{x,HCO_3^-}, \quad x = (art, bl) \quad (A9-a)$$

$$C_{x,CO_2}^T = C_{x,CO_2}^F + C_{x,HCO_3^-}, \quad x = (isf, cyt, mit) \quad (A9-b)$$

$$J_{bl \leftrightarrow cyt, CO_2}^P = \lambda_{bl \leftrightarrow cyt, CO_2} (C_{bl, CO_2}^F - C_{cyt, CO_2}^F) \quad (A10-a)$$

$$J_{cyt \leftrightarrow mit, CO_2}^P = \lambda_{cyt \leftrightarrow mit, CO_2} (C_{cyt, CO_2}^F - C_{mit, CO_2}^F) \quad (A10-b)$$

The concentration of HbCO₂ can be written in terms of its saturation (1; 2) as

$$C_{x,HbCO_2} = 4 \cdot H_{rbc} \cdot C_{rbc,Hb} \cdot S_{x,HbCO_2}, \quad S_{x,HbCO_2} = \frac{K_{HbCO_2} \cdot C_{x,CO_2}^F}{1 + K_{HbCO_2} \cdot C_{x,CO_2}^F}, \quad x = (art, bl) \quad (A11-a)$$

Applying the Henderson-Hasselbalch relation (1; 2; 4), we obtain the concentrations of HCO₃⁻ in the intravascular phases as

$$C_{x,HCO_3^-} = \left[\frac{(1 - H_{rbc})}{C_{x,pl,H^+}} + \frac{H_{rbc}}{C_{x,rbc,H^+}} \right] K_{CO_2hyd} \cdot C_{x,CO_2}^F, \quad x = (art, bl) \quad (A11-b)$$

and the concentrations of HCO₃⁻ in the extravascular phases as

$$C_{x,HCO_3^-} = \frac{K_{CO_2hyd} \cdot C_{x,CO_2}^F}{C_{x,H^+}}, \quad x = (isf, cyt, mit) \quad (A11-c)$$

Substituting Eq. (A11) in Eq. (A9), we have the expressions for total CO₂ concentrations as

$$C_{x,CO_2}^T = C_{x,CO_2}^F + \frac{4 \cdot H_{rbc} \cdot C_{rbc,Hb} \cdot K_{HbCO_2} \cdot C_{x,CO_2}^F}{1 + K_{HbCO_2} \cdot C_{x,CO_2}^F} + \left[\frac{(1 - H_{rbc})}{C_{x,pl,H^+}} + \frac{H_{rbc}}{C_{x,rbc,H^+}} \right] K_{CO_2hyd} \cdot C_{x,CO_2}^F, \quad x = (art, bl) \quad (A12-a)$$

$$C_{x,CO_2}^T = C_{x,CO_2}^F + \frac{K_{CO_2hyd} \cdot C_{x,CO_2}^F}{C_{x,H^+}}, \quad x = (isf, cyt, mit) \quad (A12-b)$$

Substituting Eq. (A12) in Eq. (A8) and using the chain rule for differentiation and equilibrium condition $C_{bl,CO_2}^F = C_{isf,CO_2}^F$, we have the dynamic mass balance equations for CO₂ as

$$(V_{bl,CO_2} + V_{isf,CO_2}) \frac{dC_{bl,CO_2}^F}{dt} = Q (C_{art,CO_2}^T - C_{bl,CO_2}^T) - J_{bl \leftrightarrow cyt, CO_2}^P \quad (A13-a)$$

$$V_{cyt,CO_2} \frac{dC_{cyt,CO_2}^F}{dt} = J_{bl \leftrightarrow cyt, CO_2}^P - J_{cyt \leftrightarrow mit, CO_2}^P \quad (A13-b)$$

$$V_{mit,CO_2} \frac{dC_{mit,CO_2}^F}{dt} = J_{cyt \leftrightarrow mit,CO_2}^p + (\phi_{PYR \leftrightarrow ACoA} + \phi_{CIT \leftrightarrow AKG} + \phi_{AKG \leftrightarrow SCoA}) \quad (A13-c)$$

where the effective volumes or volumes of distributions of CO₂ in capillary blood, tissue ISF, and tissue subcellular domains (cytosol and mitochondria) (V_{bl,CO_2} , V_{isf,CO_2} , V_{cyt,CO_2} , V_{mit,CO_2}) are given by

$$V_{bl,CO_2} = V_{bl} \left(1 + \frac{4 \cdot H_{rbc} \cdot C_{rbc,Hb} \cdot K_{HbCO_2}}{\left[1 + K_{HbCO_2} \cdot C_{bl,CO_2}^F\right]^2} + \left[\frac{(1 - H_{rbc})}{C_{bl,pl,H^+}} + \frac{H_{rbc}}{C_{bl,rbc,H^+}} \right] K_{CO_2hyd} \right) \quad (A14-a)$$

$$V_{x,CO_2} = V_x \left(1 + \frac{K_{CO_2hyd}}{C_{x,H^+}} \right), \quad x = (isf, cyt, mit) \quad (A14-b)$$

Model parameters: In the dynamics mass balance equations for O₂ and CO₂, $C_{rbc,Hb} = 5.2$ mM is the concentration of hemoglobin in RBC, $H_{rbc} = 0.45$ is the fraction of RBC in blood (hematocrits), $n_H = 2.7$ is the Hill coefficient for HbO₂ saturation, $K_{HbO_2} = 7800.7$ mM^{-2.7} is the Hill constant for HbO₂ saturation, $C_{cl,Mb} = H_{mit} \cdot C_{mit,Mb} = 0.5$ mM is the concentration of myoglobin in tissue cells, $H_{mit} = 0.1$ is the fraction of mitochondria in tissue cells, $K_{MbO_2} = 308.64$ mM⁻¹ is the Hill constant for MbO₂ saturation, $K_{HbCO_2} = 0.1237$ mM⁻¹ is the Hill constant for HbCO₂ saturation, and $K_{CO_2hyd} = 7.95E-04$ mM is the equilibrium constant for CO₂ hydration reaction (1-3). The pH in capillary blood ($pH_{bl} = pH_{pl} = pH_{rbc} = pH_{isf}$), cytosol, and mitochondria are computed through the dynamic mass balance equations for protons in these subdomains.

APPENDIX B: DYNAMIC MASS BALANCE EQUATIONS

Capillary Blood + Tissue ISF Domain

$$B1. \quad (V_{bl} + V_{isf}) \frac{dC_{bl,Glc}}{dt} = Q(C_{art,Glc} - C_{bl,Glc}) - J_{bl \leftrightarrow cyt,Glc}^f$$

$$B2. \quad (V_{bl} + V_{isf}) \frac{dC_{bl,Pyr}}{dt} = Q(C_{art,Pyr} - C_{bl,Pyr}) - J_{bl \leftrightarrow cyt,Pyr}^f$$

$$B3. \quad (V_{bl} + V_{isf}) \frac{dC_{bl,Lac}}{dt} = Q(C_{art,Lac} - C_{bl,Lac}) - J_{bl \leftrightarrow cyt,Lac}^f$$

$$B4. \quad (V_{bl} + V_{isf}) \frac{dC_{bl,Ala}}{dt} = Q(C_{art,Ala} - C_{bl,Ala}) - J_{bl \leftrightarrow cyt,Ala}^p$$

$$B5. \quad (V_{bl} + V_{isf}) \frac{dC_{bl,Glr}}{dt} = Q(C_{art,Glr} - C_{bl,Glr}) - J_{bl \leftrightarrow cyt,Glr}^p$$

$$B6. \quad (V_{bl} + V_{isf}) \frac{dC_{bl,FFA}}{dt} = Q(C_{art,FFA} - C_{bl,FFA}) - J_{bl \leftrightarrow cyt, FFA}^f$$

$$B7. \quad (V_{bl,CO2} + V_{isf,CO2}) \frac{dC_{bl,CO2}^F}{dt} = Q(C_{art,CO2}^T - C_{bl,CO2}^T) - J_{bl \leftrightarrow cyt, CO2}^p$$

$$B8. \quad (V_{bl,O2} + V_{isf,O2}) \frac{dC_{bl,O2}^F}{dt} = Q(C_{art,O2}^T - C_{bl,O2}^T) - J_{bl \leftrightarrow cyt, O2}^p$$

$$B9. \quad (V_{bl} + V_{isf}) \frac{dC_{bl,H^+}}{dt} = Q(C_{art,H^+} - C_{bl,H^+}) - J_{bl \leftrightarrow cyt, H^+}^f$$

Tissue Cells Cytosolic Domain:

$$C1. \quad V_{cyt} \frac{dC_{cyt,Glc}}{dt} = J_{bl \leftrightarrow cyt, Glc}^f - \phi_{Glc \leftrightarrow G6P}$$

$$C2. \quad V_{cyt} \frac{dC_{cyt,Pyr}}{dt} = J_{bl \leftrightarrow cyt, Pyr}^f - J_{cyt \leftrightarrow mit, Pyr}^f + \phi_{PEP \leftrightarrow Pyr} - \phi_{Pyr \leftrightarrow Lac} - \phi_{Pyr \leftrightarrow Ala}$$

$$C3. \quad V_{cyt} \frac{dC_{cyt,Lac}}{dt} = J_{bl \leftrightarrow cyt, Lac}^f + \phi_{Pyr \leftrightarrow Lac}$$

$$C4. \quad V_{cyt} \frac{dC_{cyt,Ala}}{dt} = J_{bl \leftrightarrow cyt, Ala}^p + \phi_{Pyr \leftrightarrow Ala}$$

$$C5. \quad V_{cyt} \frac{dC_{cyt,Glr}}{dt} = J_{bl \leftrightarrow cyt, Glr}^p + \phi_{Tgl \leftrightarrow Glr}$$

$$C6. \quad V_{cyt} \frac{dC_{cyt,FFA}}{dt} = J_{bl \leftrightarrow cyt, FFA}^f + 3\phi_{Tgl \leftrightarrow Glr} - \phi_{FFA \leftrightarrow FAC}$$

$$C7. \quad V_{cyt,CO2} \frac{dC_{cyt,CO2}^F}{dt} = J_{bl \leftrightarrow cyt, CO2}^p - J_{cyt \leftrightarrow mit, CO2}^p$$

$$C8. \quad V_{cyt,O2} \frac{dC_{cyt,O2}^F}{dt} = J_{bl \leftrightarrow cyt, O2}^p - J_{cyt \leftrightarrow mit, O2}^p$$

$$C9. \quad V_{cyt} \frac{dC_{cyt,Gly}}{dt} = \phi_{G6P \leftrightarrow Gly} - \phi_{Gly \leftrightarrow G6P}$$

$$C10. \quad V_{cyt} \frac{dC_{cyt,G6P}}{dt} = \phi_{Glc \leftrightarrow G6P} - \phi_{G6P \leftrightarrow Gly} + \phi_{Gly \leftrightarrow G6P} - \phi_{G6P \leftrightarrow F6P}$$

$$C11. \quad V_{cyt} \frac{dC_{cyt,F6P}}{dt} = \phi_{G6P \leftrightarrow F6P} - \phi_{F6P \leftrightarrow F16BP}$$

$$C12. V_{cyt} \frac{dC_{cyt,F16BP}}{dt} = \phi_{F6P \leftrightarrow F16BP} - \phi_{F16BP \leftrightarrow GA3P}$$

$$C13. V_{cyt} \frac{dC_{cyt,GA3P}}{dt} = 2\phi_{F16BP \leftrightarrow GA3P} - \phi_{GA3P \leftrightarrow Gr3P} - \phi_{GA3P \leftrightarrow 13BPG}$$

$$C14. V_{cyt} \frac{dC_{cyt,13BPG}}{dt} = \phi_{GA3P \leftrightarrow 13BPG} - \phi_{13BPG \leftrightarrow PEP}$$

$$C15. V_{cyt} \frac{dC_{cyt,PEP}}{dt} = \phi_{13BPG \leftrightarrow PEP} - \phi_{PEP \leftrightarrow Pyr}$$

$$C16. V_{cyt} \frac{dC_{cyt,Tgl}}{dt} = \phi_{Gr3P \leftrightarrow Tgl} - \phi_{Tgl \leftrightarrow Glr}$$

$$C17. V_{cyt} \frac{dC_{cyt,Gr3P}}{dt} = \phi_{GA3P \leftrightarrow Gr3P} - \phi_{Gr3P \leftrightarrow Tgl}$$

$$C18. V_{cyt} \frac{dC_{cyt,FAC}}{dt} = -J_{cyt \leftrightarrow mit, FAC}^f + \phi_{FFA \leftrightarrow FAC} - 3\phi_{Gr3P \leftrightarrow Tgl}$$

$$C19. V_{cyt} \frac{dC_{cyt,PCr}}{dt} = -\phi_{PCr \leftrightarrow Cr} = -V_{cyt} \frac{dC_{cyt,PCr}}{dt}$$

$$C20. V_{cyt} \frac{dC_{cyt,ATP}}{dt} = \left(-J_{cyt \leftrightarrow mit, ATP/ADP}^f - \phi_{G6P \leftrightarrow Gly} - \phi_{Glc \leftrightarrow G6P} - \phi_{F6P \leftrightarrow F16BP} + \phi_{13BPG \leftrightarrow PEP} \right. \\ \left. + \phi_{PEP \leftrightarrow Pyr} - 2\phi_{FFA \leftrightarrow FAC} - \phi_{ATP \leftrightarrow ADP} + \phi_{PCr \leftrightarrow Cr} - \phi_{AMP \leftrightarrow ADP} \right) = -V_{cyt} \frac{dC_{cyt,ADP}}{dt}$$

$$C21. V_{cyt} \frac{dC_{cyt,AMP}}{dt} = -\phi_{AMP \leftrightarrow ADP}$$

$$C22. V_{cyt} \frac{dC_{cyt,Pi}}{dt} = -J_{cyt \leftrightarrow mit, Pi}^f - \phi_{Gly \leftrightarrow G6P} + 2\phi_{G6P \leftrightarrow Gly} - \phi_{GA3P \leftrightarrow 13BPG} + \phi_{Gr3P \leftrightarrow Tgl} + 2\phi_{FFA \leftrightarrow FAC} + \phi_{ATP \leftrightarrow ADP}$$

$$C23. V_{cyt} \frac{dC_{cyt,CoA}}{dt} = -J_{cyt \leftrightarrow mit, CoA}^f + 3\phi_{Gr3P \leftrightarrow Tgl} - \phi_{FFA \leftrightarrow FAC}$$

$$C24. V_{cyt} \frac{dC_{cyt,NADH}}{dt} = -J_{cyt \leftrightarrow mit, NADH/NAD^+}^f + \phi_{GA3P \leftrightarrow 13BPG} - \phi_{Pyr \leftrightarrow Lac} - \phi_{GA3P \leftrightarrow Gr3P} = -V_{cyt} \frac{dC_{cyt,NAD^+}}{dt}$$

$$C25. V_{cyt} \frac{dC_{cyt,H^+}}{dt} = \left(\begin{array}{c} J_{bl \leftrightarrow cyt, H^+}^f \\ -J_{cyt \leftrightarrow mit, H^+}^f \end{array} \right) + \frac{2.303}{\beta_{cyt}} C_{cyt, H^+} \left(\begin{array}{c} +\phi_{Glc \leftrightarrow G6P} + \phi_{F6P \leftrightarrow F16BP} + \phi_{GA3P \leftrightarrow 13BPG} \\ -\phi_{PEP \leftrightarrow Pyr} - \phi_{Pyr \leftrightarrow Lac} + 3\phi_{Tgl \leftrightarrow Glr} - \phi_{GA3P \leftrightarrow Gr3P} \\ +2\phi_{FFA \leftrightarrow FAC} + \phi_{ATP \leftrightarrow ADP} - \phi_{PCr \leftrightarrow Cr} - J_{cyt \leftrightarrow mit, H^+}^{leak} \end{array} \right)$$

where $\beta_{cyt} = 6.65 \text{ mM/pH}$ is buffering capacity of cytosol for protons

Tissue Cells Mitochondrial Domain:

$$M1. \quad V_{mit,O_2} \frac{dC_{mit,O_2}^F}{dt} = J_{cyt \leftrightarrow mit,O_2}^P - 0.5\phi_{O_2 \leftrightarrow H_2O,NADH} - 0.5\phi_{O_2 \leftrightarrow H_2O,FADH_2}$$

$$M2. \quad V_{mit,CO_2} \frac{dC_{mit,CO_2}^F}{dt} = J_{cyt \leftrightarrow mit,CO_2}^P + (\phi_{Pyr \leftrightarrow ACoA} + \phi_{Cit \leftrightarrow AKG} + \phi_{AKG \leftrightarrow SCoA})$$

$$M3. \quad V_{mit} \frac{dC_{mit,Pyr}}{dt} = J_{cyt \leftrightarrow mit,Pyr}^f - \phi_{Pyr \leftrightarrow ACoA}$$

$$M4. \quad V_{mit} \frac{dC_{mit,FAC}}{dt} = J_{cyt \leftrightarrow mit,FAC}^f - \phi_{FAC \leftrightarrow ACoA}$$

$$M5. \quad V_{mit} \frac{dC_{mit,NADH}}{dt} = \left(J_{cyt \leftrightarrow mit,NADH/NAD^+}^f + \phi_{Pyr \leftrightarrow ACoA} + 7\phi_{FAC \leftrightarrow ACoA} + \phi_{Cit \leftrightarrow AKG} \right) + \phi_{AKG \leftrightarrow SCoA} + \phi_{Mal \leftrightarrow Oxa} - \phi_{O_2 \leftrightarrow H_2O,NADH} = -V_{mit} \frac{dC_{mit,NAD^+}}{dt}$$

$$M6. \quad V_{mit} \frac{dC_{mit,ATP}}{dt} = -J_{cyt \leftrightarrow mit,ATP/ADP}^f + \phi_{SCoA \leftrightarrow Suc} + \phi_{ADP \leftrightarrow ATP} = -V_{mit} \frac{dC_{mit,ADP}}{dt}$$

$$M7. \quad V_{mit} \frac{dC_{mit,Pi}}{dt} = J_{cit \leftrightarrow mit,Pi}^f - \phi_{SCoA \leftrightarrow Suc} - \phi_{ADP \leftrightarrow ATP}$$

$$M8. \quad V_{mit} \frac{dC_{mit,CoA}}{dt} = J_{cyt \leftrightarrow mit,CoA}^f - \phi_{Pyr \leftrightarrow ACoA} - 7\phi_{FAC \leftrightarrow ACoA} + \phi_{ACoA \leftrightarrow Cit} - \phi_{AKG \leftrightarrow SCoA} + \phi_{SCoA \leftrightarrow Suc}$$

$$M9. \quad V_{mit} \frac{dC_{mit,ACoA}}{dt} = \phi_{Pyr \leftrightarrow ACoA} + 8\phi_{FAC \leftrightarrow ACoA} - \phi_{ACoA \leftrightarrow Cit}$$

$$M10. \quad V_{mit} \frac{dC_{mit,Cit}}{dt} = \phi_{ACoA \leftrightarrow Cit} - \phi_{Cit \leftrightarrow AKG}$$

$$M11. \quad V_{mit} \frac{dC_{mit,AKG}}{dt} = \phi_{Cit \leftrightarrow AKG} - \phi_{AKG \leftrightarrow SCoA}$$

$$M12. \quad V_{mit} \frac{dC_{mit,SCoA}}{dt} = \phi_{AKG \leftrightarrow SCoA} - \phi_{SCoA \leftrightarrow Suc}$$

$$M13. \quad V_{mit} \frac{dC_{mit,Suc}}{dt} = \phi_{SCoA \leftrightarrow Suc} - \phi_{Suc \leftrightarrow Mal}$$

$$M14. \quad V_{mit} \frac{dC_{mit,Mal}}{dt} = \phi_{Suc \leftrightarrow Mal} - \phi_{Mal \leftrightarrow Oxa}$$

$$M15. \quad V_{mit} \frac{dC_{mit,Oxa}}{dt} = \phi_{Mal \leftrightarrow Oxa} - \phi_{ACoA \leftrightarrow Cit} = -V_{mus} \frac{d}{dt} [C_{mit,Cit} + C_{mit,AKG} + C_{mit,SCoA} + C_{mit,Suc} + C_{mit,Mal}]$$

$$\text{M16. } V_{mit} \frac{dC_{mit,FADH2}}{dt} = 7\phi_{FAC \leftrightarrow ACoA} + \phi_{Suc \leftrightarrow Mal} - \phi_{O2 \rightarrow H2O, FADH2} = -V_{mit} \frac{dC_{mit,FAD}}{dt}$$

$$\text{M17. } V_{mit} \frac{dC_{mit,H^+}}{dt} = J_{cyt \leftrightarrow mit, H^+}^f + \frac{2.303}{\beta_{mit}} C_{mit,H^+} \left(\begin{array}{l} J_{cyt \leftrightarrow mit, H^+}^{leak} + \phi_{Pyr \leftrightarrow ACoA} + 7\phi_{FAC \leftrightarrow ACoA} + \phi_{ACoA \leftrightarrow Cit} + \phi_{Mal \leftrightarrow Oxa} \\ -(10+1)\phi_{O2 \rightarrow H2O, NADH} - 6\phi_{O2 \rightarrow H2O, FADH2} + (3-1)\phi_{ADP \leftrightarrow ATP} \end{array} \right)$$

where $\beta_{mit} = 25$ mM/pH is buffering capacity of mitochondria for protons

$$\text{M18. } C_{IMM} \frac{d\Delta\Psi}{dt} = 10\phi_{O2 \rightarrow H2O, NADH} + 6\phi_{O2 \rightarrow H2O, FADH2} - 3\phi_{ADP \leftrightarrow ATP} - J_{cyt \leftrightarrow mit, H^+}^{leak}$$

where $C_{IMM} = 6.75 \cdot 10^{-3}$ mmol/mV is the capacitance of the IMM

APPENDIX C: RESTING STEADY-STATE FLUX RELATIONSHIPS

By setting the time derivatives of the mass balances (Appendix B) equal to zero, we can obtain the resting steady-state flux relationships. The fluxes from these relationships can be arranged for sequential solution as shown in the table below in which the independently known fluxes (in parentheses) are listed in Tables 3. With a few exceptions, these relationships are obtained directly from the mass balances indicated in the right column. An additional relationship is used for the metabolic reaction flux associated with ATP synthase in mitochondria:

$$\phi_{mit,ADP \leftrightarrow ATP}^0 = 2.5\phi_{O_2 \leftrightarrow H_2O, NADH}^0 + 1.5\phi_{O_2 \leftrightarrow H_2O, FADH_2}^0 \quad (\text{C1})$$

The basis for this relationship is that in oxidative phosphorylation, 1 mole of NADH can produce 2.5 moles of ATP and 1 mole FADH₂ can produce 1.5 moles ATP.

To compute the ATPase in cytosol, we note that at rest, the steady-state metabolic fluxes associated with adenylate kinase and creatine kinase are zero:

$$\phi_{PCr \leftrightarrow Cr}^0 = 0 \quad \text{and} \quad \phi_{AMP \leftrightarrow ADP}^0 = 0 \quad (\text{C2})$$

Therefore, the steady-state metabolic flux associated with ATPase in cytosol (C20, Appendix B) can be simplified to

$$\begin{aligned} f_{cyt,ATP \leftarrow ADP}^0 &= f_{13BPG \leftarrow PEP}^0 + f_{PEP \leftarrow Pyr}^0 - f_{Glc \leftarrow G6P}^0 - f_{G6P \leftarrow Gly}^0 \\ &\quad - f_{F6P \leftarrow F16BP}^0 - 2f_{FFA \leftarrow FAC}^0 - J_{c \leftarrow m,ATP/ADP}^{f,0} \end{aligned} \quad (\text{C3})$$

Metabolic Reactions	Flux Relations Arranged for Sequential Solution	Mass Balance (Appendix B)
Hexokinase	$f_{Glc \leftarrow G6P}^0 = J_{bc, Glc}^{f,0}$	C1
Phosphoglucose isomerase	$f_{G6P \leftarrow F6P}^0 = f_{Glc \leftarrow G6P}^0$	C10

<i>Phosphofructokinase</i>	$f_{F6P \ll F16BP}^0 = f_{G6P \ll F6P}^0$	C11
<i>Aldolase + TPI</i>	$f_{F16BP \ll GA3P}^0 = f_{Glc \ll G6P}^0$	C12
<i>Lipases</i>	$f_{Tgl \ll Glr}^0 = -J_{b \ll c, Glr}^{p,0}$	C5
<i>Acyltransferase</i>	$f_{Gr3P \ll Tgl}^0 = f_{Tgl \ll Glr}^0$	C16
<i>Gr3P dehydrogenase</i>	$f_{GA3P \ll Gr3P}^0 = f_{Gr3P \ll Tgl}^0$	C17
<i>GA3P dehydrogenase</i>	$f_{GA3P \ll 13BPG}^0 = 2f_{F16BP \ll GA3P}^0 - f_{GA3P \ll Gr3P}^0$	C13
<i>Phosphoglycerate kinase</i>	$f_{13BPG \ll PEP}^0 = f_{GA3P \ll 13BPG}^0$	C14
<i>Pyruvate kinase</i>	$f_{PEP \ll Pyr}^0 = f_{13BPG \ll PEP}^0$	C15
<i>Lactate dehydrogenase</i>	$f_{Pyr \ll Lac}^0 = -J_{b \ll c, Lac}^{f,0}$	C3
<i>Alanine aminotransferase</i>	$f_{Pyr \ll Ala}^0 = -J_{b \ll c, Ala}^{p,0}$	C4
<i>Acyl-CoA synthetase</i>	$f_{FFA \ll FAC}^0 = J_{b \ll c, FFA}^{f,0} + 3f_{Tgl \ll Glr}^0$	C6
<i>c\leftrightarrowm Pyruvate transport</i>	$J_{c \ll m, Pyr}^{f,0} = f_{PEP \ll Pyr}^0 - f_{Pyr \ll Lac}^0 - f_{Pyr \ll Ala}^0 + J_{b \ll c, Pyr}^{f,0}$	C2
<i>c\leftrightarrowm Fatty Acyl-CoA transport</i>	$J_{c \ll m, FAC}^{f,0} = f_{FFA \ll FAC}^0 - 3f_{Gr3P \ll Tgl}^0$	C18
<i>Pyruvate dehydrogenase</i>	$f_{Pyr \ll ACoA}^0 = J_{c \ll m, Pyr}^{f,0}$	M3
<i>Beta oxidation</i>	$f_{FAC \ll ACoA}^0 = J_{c \ll m, FAC}^{f,0}$	M4
<i>Citrate synthase</i>	$f_{ACoA \ll Cit}^0 = f_{Pyr \ll ACoA}^0 + 8f_{FAC \ll ACoA}^0$	M9
<i>Isocitrate dehydrogenase</i>	$f_{Cit \ll AKG}^0 = f_{ACoA \ll Cit}^0$	M10
<i>AKG dehydrogenase</i>	$f_{AKG \ll SCoA}^0 = f_{Cit \ll AKG}^0$	M11
<i>SCoA synthetase</i>	$f_{SCoA \ll Suc}^0 = f_{AKG \ll SCoA}^0$	M12
<i>Succinate dehydrogenase</i>	$f_{Suc \ll Mal}^0 = f_{SCoA \ll Suc}^0$	M13
<i>Malate dehydrogenase</i>	$f_{Mal \ll Oxa}^0 = f_{Suc \ll Mal}^0$	M14
<i>c\leftrightarrowm NADH/NAD⁺ transport</i>	$J_{c \ll m, NADH/NAD^+}^{f,0} = f_{GA3P \ll 13BPG}^0 - f_{Pyr \ll Lac}^0 - f_{GA3P \ll Gr3P}^0$	C24
<i>Oxidative phosphorylation (NADH)</i>	$f_{O_2 \ll H_2O, NADH}^0 = f_{Pyr \ll ACoA}^0 + f_{Cit \ll AKG}^0 + f_{AKG \ll SCoA}^0 + f_{Mal \ll Oxa}^0 + 7f_{FAC \ll ACoA}^0 + J_{c \ll m, NADH/NAD^+}^{f,0}$	M5
<i>Oxidative phosphorylation (FADH₂)</i>	$f_{O_2 \ll H_2O, FADH_2}^0 = f_{Suc \ll Mal}^0 + 7f_{FAC \ll ACoA}^0$	M16

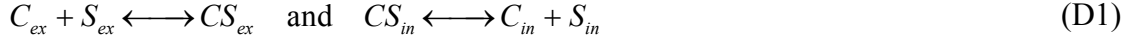
<i>ATPase (ATP synthesis)</i>	$\phi_{m,ADP \leftrightarrow ATP}^0 = 2.5\phi_{O_2 \leftrightarrow H_2O, NADH}^0 + 1.5\phi_{O_2 \leftrightarrow H_2O, FADH_2}^0$	
<i>c ↔ m ATP/ADP transport</i>	$J_{c \leftarrow m, ATP/ADP}^{f,0} = - (f_{ADP \leftarrow ATP}^0 + f_{SCoA \leftarrow Suc}^0)$	M6
<i>ATPase (ATP hydrolysis)</i>	$f_{c, ATP \leftarrow ADP}^0 = f_{13BPG \leftarrow PEP}^0 + f_{PEP \leftarrow Pyr}^0 - f_{Glc \leftarrow G6P}^0$ $- f_{G6P \leftarrow Gly}^0 - f_{F6P \leftarrow F16BP}^0 - 2f_{FFA \leftarrow FAC}^0 - J_{c \leftarrow m, ATP/ADP}^{f,0}$	C20
<i>c ↔ m Pi transport</i>	$J_{c \leftarrow m, Pi}^{f,0} = f_{ADP \leftarrow ATP}^0 + f_{SCoA \leftarrow Suc}^0$	M7#
<i>c ↔ m CoA transport</i>	$J_{c \leftarrow m, CoA}^{f,0} = f_{AKG \leftarrow SCoA}^0 + 7f_{FAC \leftarrow ACoA}^0 + f_{Pyr \leftarrow ACoA}^0$ $- f_{ACoA \leftarrow Cit}^0 - f_{SCoA \leftarrow Suc}^0$	M8*
<i>c ↔ m O₂ transport</i>	$J_{c \leftarrow m, O_2}^{f,0} = 0.5 * (f_{O_2 \leftarrow H_2O, NADH}^0 + f_{O_2 \leftarrow H_2O, FADH_2}^0)$	M1##
<i>c ↔ m CO₂ transport</i>	$J_{c \leftarrow m, CO_2}^{f,0} = - (f_{Pyr \leftarrow ACoA}^0 + f_{Cit \leftarrow AKG}^0 + f_{AKG \leftarrow SCoA}^0)$	M2**
<i>c ↔ m H⁺ leak</i>	$J_{c \leftarrow m, H^+}^{leak,0} = 10f_{O_2 \leftarrow H_2O, NADH}^0 + 6f_{O_2 \leftarrow H_2O, FADH_2}^0 - 3f_{ADP \leftarrow ATP}^0$	M18
<i>c ↔ m H⁺ transport</i>	$J_{c \leftarrow m, H^+}^{f,0} = - \frac{2.303}{\beta_{mit}} C_{mit, H^+}^0 (\phi_{Pyr \leftrightarrow ACoA}^0 + 7\phi_{FAC \leftrightarrow ACoA}^0$ $+ \phi_{ACoA \leftrightarrow Cit}^0 + \phi_{Mal \leftrightarrow Oxa}^0 - \phi_{O_2 \rightarrow H_2O, NADH}^0 - \phi_{ADP \leftrightarrow ATP}^0)$	M17
<i>b ↔ c H⁺ transport</i>	$J_{b \leftrightarrow c, H^+}^{f,0} = J_{c \leftarrow m, H^+}^{f,0} - \frac{2.303}{\beta_{cyt}} C_{cyt, H^+}^0 (+\phi_{Glc \leftrightarrow G6P}^0 + \phi_{F6P \leftrightarrow F16BP}^0$ $+ \phi_{GA3P \leftrightarrow 13BPG}^0 - \phi_{PEP \leftrightarrow Pyr}^0 - \phi_{Pyr \leftrightarrow Lac}^0 + 3\phi_{Tgl \leftrightarrow Glr}^0 - \phi_{GA3P \leftrightarrow Gr3P}^0$ $+ 2\phi_{FFA \leftrightarrow FAC}^0 + \phi_{ATP \leftrightarrow ADP}^0 - J_{c \leftarrow m, H^+}^{leak,0})$	C25

c ↔ m: *cyt ↔ mit* or *cytosol ↔ mitochondria*; *b ↔ c*: *bl ↔ cyt* or *blood ↔ cytosol*
#*c ↔ m* Pi transport can also be obtained from Eq. (C22); but it will give the same result
**c ↔ m* CoA transport can also be obtained from Eq. (C23); but it will give the same result
##*b ↔ c* O₂ transport flux at rest = *c ↔ m* O₂ transport flux at rest (Eq. C8).
***b ↔ c* CO₂ transport flux at rest = *c ↔ m* CO₂ transport flux at rest (Eq. C7).

APPENDIX D: GENERAL FLUX EXPRESSION FOR FACILITATED TRANSPORT

In a carrier-mediated (facilitated) transport of uncharged substrate molecules (e.g., glucose, lactate, pyruvate, free fatty acid) through a biological membrane (e.g., sarcolemma), a substrate molecule S first binds with a carrier molecule C to form a molecule of the carrier-substrate complex CS which then diffuses passively (freely) through the membrane. Simultaneously, the carrier molecules used to shuttle the substrate molecules into the cells rediffuse freely from intracellular side to extracellular side of the membrane (see Reference (9), Chapter 2). Such facilitated

substrate transport process can be considered to be in pseudo-steady state. The associated chemical reactions can be expressed as



The binding of the substrate molecules (S) with the carrier molecules (C) is usually fast so that the chemical equilibrium condition can be assumed to prevail. This gives

$$M_{ex}[CS]_{ex} = [C]_{ex}[S]_{ex} \quad \text{and} \quad M_{in}[CS]_{in} = [C]_{in}[S]_{in} \quad (\text{D2})$$

where M_{ex} and M_{in} are equilibrium constants. Furthermore, for a closed system:

$$[CS]_{ex} + [CS]_{in} + [C]_{in} + [C]_{ex} = [C]_{tot} \quad (\text{D3})$$

The transport fluxes of the carrier-substrate complex (CS) and free carrier (C) through the membrane are based on passive diffusion (Fick's principle):

$$J_{ex \leftrightarrow in}^{CS} = \frac{D_{CS}}{d_m} ([CS]_{ex} - [CS]_{in}) \quad \text{and} \quad J_{ex \leftrightarrow in}^C = \frac{D_C}{d_m} ([C]_{in} - [C]_{ex}) \quad (\text{D4})$$

where D_{CS} and D_C are molecular diffusion coefficients and d_m is thickness of the membrane. Under steady-state conditions, the transport fluxes are equal:

$$J_{ex \leftrightarrow in} = J_{ex \leftrightarrow in}^{CS} = J_{ex \leftrightarrow in}^C \quad \text{or} \quad \frac{D_{CS}}{d_m} ([CS]_{ex} - [CS]_{in}) = \frac{D_C}{d_m} ([C]_{in} - [C]_{ex}) \quad (\text{D5})$$

The system of equations (D2), (D3) and (D5) is equivalent to

$$\begin{aligned} \frac{D_{CS}}{d_m} \left(\frac{[C]_{ex}[S]_{ex}}{M_{ex}} - \frac{[C]_{in}[S]_{in}}{M_{in}} \right) - \frac{D_C}{d_m} ([C]_{in} - [C]_{ex}) &= 0 \\ \frac{[C]_{ex}[S]_{ex}}{M_{ex}} + \frac{[C]_{in}[S]_{in}}{M_{in}} + [C]_{in} + [C]_{ex} &= [C]_{tot} \end{aligned} \quad (\text{D6})$$

These can be solved simultaneously to obtain the free carrier concentrations $[C]_{ex}$ and $[C]_{in}$. Using the reasonable assumptions $D_{CS} = D_C = D$ and $M_{in} = M_{ex} = M_m$, the system of equations (D6) can be solved to yield

$$[C]_{ex} = \frac{M_m \cdot [C]_{tot}}{2(M_m + [S]_{ex})} \quad \text{and} \quad [C]_{in} = \frac{M_m \cdot [C]_{tot}}{2(M_m + [S]_{in})} \quad (\text{D7})$$

Substituting equations (D7) into equations (D5), we have the reversible carrier-mediated facilitated transport flux given by

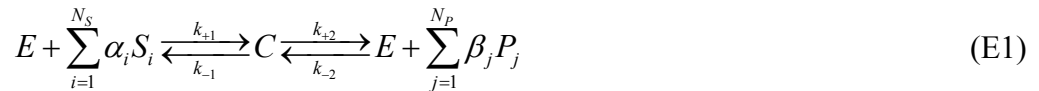
$$J_{ex \leftrightarrow in} = T_{\max} \left(\frac{M_m}{M_m + [S]_{in}} - \frac{M_m}{M_m + [S]_{ex}} \right) = T_{\max} \left(\frac{[S]_{ex}}{M_m + [S]_{ex}} - \frac{[S]_{in}}{M_m + [S]_{in}} \right) \quad (\text{D8})$$

where $T_{\max} = D[C]_{\text{tot}}/2d_m$ is the maximal transport rate of carrier-mediated facilitated transport. The flux expression (D8) is of the form of Michaelis-Menten equation for enzyme kinetics and satisfies thermodynamic equilibrium conditions $[S]_{\text{ex}} = [S]_{\text{in}}$.

The transport of glucose, lactate, pyruvate, free fatty acid, and H^+ across the sarcolemma (cell membrane) and the transport of pyruvate, fatty acyl-CoA, CoA, Pi, and H^+ across the inner mitochondrial membrane are considered to be carrier-mediated (facilitated) transport. The anti-transport of ATP and ADP across the inner mitochondrial membrane occurs through the adenine nucleotide translocase (ANT) and is phenomenologically modeled here via a facilitated transport flux expression in terms of the phosphorylation potential $[\text{ATP}]/[\text{ADP}]$. The hypothetical anti-transport flux of NADH and NAD^+ across the inner mitochondrial membrane, which is proportional to the effective flux through the malate-aspartate and glycerolphosphate shuttle systems, is phenomenologically modeled here via a facilitated transport flux expression in terms of the redox potential $[\text{NADH}]/[\text{NAD}^+]$. The inter-domain transport of all other species is considered to be through passive diffusion.

APPENDIX E: GENERAL FLUX EXPRESSION FOR METABOLIC REACTIONS

Consider a general multi-reactant multi-product enzymatic reaction which incorporates several elementary enzymatic reactions. The enzyme “E” may represent a “hypothetical” multi-enzyme complex. The kinetics of this lumped enzymatic reaction is highly complex (8). For simplicity and for developing a framework for large-scale computational models, we assume here that the reaction is irreversible and proceeds in a single step as represented by



where S_i 's are the reactants, P_j 's are the products, C is an intermediate complex that incorporates the enzyme E, α_i 's and β_j 's are the stoichiometric coefficients, and k 's are the rate constants. Since the total enzyme concentration is constant, we get the stoichiometric relationship: $[E]_{\text{tot}} = [C] + [E]$, which can be applied to reduce the number of state variables. The rates of production and utilization of the complex C are given by

$$P(C) = k_{+1}[E] \prod_{i=1}^{N_S} [S_i]^{\alpha_i} + k_{-2}[E] \prod_{j=1}^{N_P} [P_j]^{\beta_j}, \quad U(C) = k_{-1}[C] + k_{+2}[C] \quad (\text{E2})$$

Now considering a quasi-steady state approximation for the complex C (see Reference (8), Chapter 2), we have

$$\frac{d[C]}{dt} = P(C) - U(C) = 0 \Rightarrow k_{+1}[E] \prod_{i=1}^{N_S} [S_i]^{\alpha_i} + k_{-2}[E] \prod_{j=1}^{N_P} [P_j]^{\beta_j} = k_{-1}[C] + k_{+2}[C] \quad (\text{E3})$$

which can be rearranged to give

$$[C] = \frac{k_{+1} \prod_{i=1}^{N_S} [S_i]^{\alpha_i} + k_{-2} \prod_{j=1}^{N_P} [P_j]^{\beta_j}}{k_{-1} + k_{+2}} [E] \quad (\text{E4})$$

The net reaction velocity or flux in the direction of product formation is given by

$$\phi_{net} = \phi_f - \phi_b = k_{+2}[C] - k_{-2}[E] \prod_{j=1}^{N_P} [P_j]^{\beta_j} = \frac{k_{+1}k_{+2} \prod_{i=1}^{N_S} [S_i]^{\alpha_i} - k_{-1}k_{-2} \prod_{j=1}^{N_P} [P_j]^{\beta_j}}{k_{-1} + k_{+2}} [E] \quad (\text{E5})$$

Dividing Eq. (E5) by $[E]_{tot}$, and using the stoichiometric relationship, we have

$$\frac{\phi_{net}}{[E]_{tot}} = \frac{\phi_f - \phi_b}{[E]_{tot}} = \frac{k_{+1}k_{+2} \prod_{i=1}^{N_S} [S_i]^{\alpha_i} - k_{-1}k_{-2} \prod_{j=1}^{N_P} [P_j]^{\beta_j}}{k_{-1} + k_{+2}} \frac{[E]}{[C] + [E]} \quad (\text{E6})$$

Substituting Eq. (E4) for $[C]$ into Eq. (E6), we have

$$\phi_{net} = \phi_f - \phi_b = \frac{k_{+1}k_{+2} \prod_{i=1}^{N_S} [S_i]^{\alpha_i} - k_{-1}k_{-2} \prod_{j=1}^{N_P} [P_j]^{\beta_j}}{k_{-1} + k_{+2} + k_{+1} \prod_{i=1}^{N_S} [S_i]^{\alpha_i} + k_{-2} \prod_{j=1}^{N_P} [P_j]^{\beta_j}} [E]_{tot} \quad (\text{E7})$$

Now, if we define the parameters

$$V_{max}^f = k_{+2}[E]_{tot}; \quad V_{max}^b = k_{-1}[E]_{tot}; \quad K_m^S = \frac{k_{-1} + k_{+2}}{k_{+1}}; \quad K_m^P = \frac{k_{-1} + k_{+2}}{k_{-2}} \quad (\text{E8})$$

then Eq. (E8) for net velocity or flux of the reaction has the following form:

$$\phi_{net} = \phi_f - \phi_b = \left[\frac{V_{max}^f}{K_m^S} \prod_{i=1}^{N_S} [S_i]^{\alpha_i} - \frac{V_{max}^b}{K_m^P} \prod_{j=1}^{N_P} [P_j]^{\beta_j} \right] \left[1 + \frac{1}{K_m^S} \prod_{i=1}^{N_S} [S_i]^{\alpha_i} + \frac{1}{K_m^P} \prod_{j=1}^{N_P} [P_j]^{\beta_j} \right]^{-1} \quad (\text{E9})$$

To be thermodynamically feasible, the flux expression (E9) must satisfy Haldane constraint relating forward and reverse velocities to equilibrium constant (Reference (8), Chapter 2):

$$\frac{V_{max}^f}{V_{max}^b} \cdot \frac{K_m^P}{K_m^S} = K_{eq}; \quad K_{eq} = \prod_{j=1}^{N_P} [P_j]_{eq}^{\beta_j} / \prod_{i=1}^{N_S} [S_i]_{eq}^{\alpha_i} = \exp(-\Delta G_0 / RT) \quad (\text{E10})$$

where K_{eq} is the equilibrium constant and ΔG_0 is the standard Gibbs free energy change.

Any multi-substrate multi-product irreversible enzymatic reaction can be considered as a special case of reaction (E1) in which the reverse reaction is considered negligible (i.e., $k_{-2} \approx 0$ or K_{eq} and K_m^P are large). The resulting forward flux expression can be derived from Eq. (E9) by setting $1/K_m^P = 0$ which is given by

$$\phi_f = V_{\max}^f \left[\frac{1}{K_m^S} \prod_{i=1}^{N_s} [S_i]^{\alpha_i} \right] \left[1 + \frac{1}{K_m^S} \prod_{i=1}^{N_s} [S_i]^{\alpha_i} \right]^{-1} \quad (\text{E11})$$

The metabolic reaction flux expressions (E9) or (E11) include the metabolic energy controller pairs ATP-ADP or ATP-AMP (phosphorylation pair) and NADH-NAD⁺ (redox pair) as the co-substrates and/or co-products. The maximal forward and reverse reaction velocities V_{\max}^f and V_{\max}^b can be further modified to account for activation and/or inhibition of the metabolic reaction flux by other metabolites in the biochemical pathways. The flux expressions for the individual lumped metabolic reactions are given in Appendix F.

APPENDIX F: METABOLIC REACTION FLUX EXPRESSIONS

Reactions in Cytosol

1. *Glycogen Phosphorylase*



$$\phi_{\text{Gly} \leftrightarrow \text{G6P}} = \left[\frac{\frac{C_{\text{AMP}}}{C_{\text{ATP}}}}{K_{\text{Gly} \leftrightarrow \text{G6P}}^{\text{Ctrl}} + \frac{C_{\text{AMP}}}{C_{\text{ATP}}}} \right] \left[\frac{V_{\text{Gly} \rightarrow \text{G6P}} \frac{C_{\text{Gly}_{n+1}} C_{\text{Pi}}}{K_{\text{Gly} \rightarrow \text{G6P}}} - V_{\text{Gly} \leftarrow \text{G6P}} \frac{C_{\text{Gly}_n} C_{\text{G6P}}}{K_{\text{Gly} \leftarrow \text{G6P}}}}{1 + \frac{C_{\text{Gly}_{n+1}} C_{\text{Pi}}}{K_{\text{Gly} \rightarrow \text{G6P}}} + \frac{C_{\text{Gly}_n} C_{\text{G6P}}}{K_{\text{Gly} \leftarrow \text{G6P}}}} \right]$$

This is a sum of 2 enzymatic reactions $\text{Gly}_{n+1} + \text{Pi} \leftrightarrow \text{Gly}_n + \text{G1P}$ and $\text{G1P} \leftrightarrow \text{G6P}$ catalyzed by the enzymes *glycogen phosphorylase* and *phosphoglucomutase* (see Ref. (7), Ch. 20; Ref. (10), Ch. 23). The activity of glycogen phosphorylase is regulated by AMP and ATP; AMP acts as a positive allosteric effector (activator) and ATP acts a negative allosteric effector (inhibitor) by competing with AMP. Thus, this reaction is assumed to be controlled by the concentration ratio $C_{\text{AMP}}/C_{\text{ATP}}$. This reaction is also known to be regulated by Ca^{2+} and epinephrine which are not explicitly included in this model of muscle metabolism.

2. *Glycogen Synthase*



$$\phi_{\text{G6P} \leftrightarrow \text{Gly}} = \left[\frac{V_{\text{G6P} \rightarrow \text{Gly}} \frac{C_{\text{Gly}_n} C_{\text{G6P}} C_{\text{ATP}}}{K_{\text{G6P} \rightarrow \text{Gly}}} - V_{\text{G1P} \leftarrow \text{Gly}} \frac{C_{\text{Gly}_{n+1}} C_{\text{Pi}}^2 C_{\text{ADP}}}{K_{\text{G6P} \leftarrow \text{Gly}}}}{1 + \frac{C_{\text{Gly}_n} C_{\text{G6P}} C_{\text{ATP}}}{K_{\text{G6P} \rightarrow \text{Gly}}} + \frac{C_{\text{Gly}_{n+1}} C_{\text{Pi}}^2 C_{\text{ADP}}}{K_{\text{G6P} \leftarrow \text{Gly}}}} \right]$$

This is a sum of 4 enzymatic reactions $G6P \leftrightarrow G1P$, $G1P + UTP \leftrightarrow UDP\text{-}Glc + 2 Pi$, $UDP\text{-}Glc + Gly_n \leftrightarrow UDP + Gly_{n+1}$, and $UDP + ATP \leftrightarrow UTP + ADP$ catalyzed by the enzymes *phosphoglucosmutase*, *UDP-Glc pyrophosphorylase*, *glycogen synthase*, and *nucleoside diphosphokinase* (see Ref. (7), Ch. 20; Ref. (10), Ch. 23). Insulin can activate the glycogen synthesis reaction. The negative allosteric regulator (inhibitor) of the reaction includes Ca^{2+} which is not explicitly included in this model of muscle metabolism.

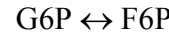
3. Hexokinase



$$\phi_{Glc \leftrightarrow G6P} = \left[\frac{K_{Glc \leftrightarrow G6P}^{Ctrl}}{K_{Glc \leftrightarrow G6P}^{Ctrl} + C_{G6P}} \right] \left[\frac{V_{Glc \rightarrow G6P} \frac{C_{Glc} C_{ATP}}{K_{Glc \rightarrow G6P}} - V_{Glc \leftarrow G6P} \frac{C_{G6P} C_{ADP} C_{H^+}}{K_{Glc \leftarrow G6P}}}{1 + \frac{C_{Glc} C_{ATP}}{K_{Glc \rightarrow G6P}} + \frac{C_{G6P} C_{ADP} C_{H^+}}{K_{Glc \leftarrow G6P}}} \right]$$

This is a sum of 2 elementary reactions $Glc + Pi \leftrightarrow G6P$ (unfavorable) and $ATP \leftrightarrow ADP + Pi + H^+$ (favorable) catalyzed by the enzyme *hexokinase* (see Ref. (7), Ch. 15; Ref. (10), Ch. 19). The enzyme is considered to be inhibited by G6P (uncompetitive).

4. Phosphoglucose Isomerase



$$\phi_{G6P \leftrightarrow F6P} = \left[\frac{V_{G6P \rightarrow F6P} \frac{C_{G6P}}{K_{G6P \rightarrow F6P}} - V_{G6P \leftarrow F6P} \frac{C_{F6P}}{K_{G6P \leftarrow F6P}}}{1 + \frac{C_{G6P}}{K_{G6P \rightarrow F6P}} + \frac{C_{F6P}}{K_{G6P \leftarrow F6P}}} \right]$$

5. Phosphofructokinase



$$\phi_{F6P \leftrightarrow F16BP} = \left[\frac{C_{AMP}}{K_{F6P \leftrightarrow F16BP}^{Ctrl} + C_{AMP}} \right] \left[\frac{V_{F6P \rightarrow F16BP} \frac{C_{F6P} C_{ATP}}{K_{F6P \rightarrow F16BP}} - V_{F6P \leftarrow F16BP} \frac{C_{F16BP} C_{ADP} C_{H^+}}{K_{F6P \leftarrow F16BP}}}{1 + \frac{C_{F6P} C_{ATP}}{K_{F6P \rightarrow F16BP}} + \frac{C_{F16BP} C_{ADP} C_{H^+}}{K_{F6P \leftarrow F16BP}}} \right]$$

The enzyme *phosphofructokinase* is the most important control element in the glycolytic pathway. This enzyme is known to be activated by AMP (see Ref. (7), Ch. 15; Ref. (10), Ch. 19).

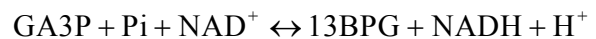
6. Glyceraldehyde 3-Phosphate Formation



$$\phi_{F16BP \leftrightarrow GA3P} = \left[\frac{V_{F16BP \rightarrow GA3P} \frac{C_{F16BP}}{K_{F16BP \rightarrow GA3P}} - V_{F16BP \leftarrow GA3P} \frac{C_{GA3P}^2}{K_{F16BP \leftarrow GA3P}}}{1 + \frac{C_{F16BP}}{K_{F16BP \rightarrow GA3P}} + \frac{C_{GA3P}^2}{K_{F16BP \leftarrow GA3P}}} \right]$$

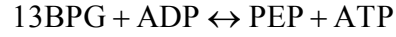
This is a sum of 2 enzymatic reactions $F16BP \leftrightarrow GA3P + DHAP$ and $DHAP \leftrightarrow GA3P$ catalyzed by the enzymes *aldolase* and *triose phosphate isomerase* (see Ref. (7), Ch. 15; Ref. (10), Ch. 19). Because these reactions are almost in equilibrium, they are combined to one reaction.

7. Glyceraldehyde 3-Phosphate Dehydrogenase



$$\phi_{\text{GA3P} \leftrightarrow \text{13BPG}} = \left[\frac{V_{\text{GA3P} \rightarrow \text{13BPG}} \frac{C_{\text{GA3P}} C_{\text{Pi}} C_{\text{NAD}^+}}{K_{\text{GA3P} \rightarrow \text{13BPG}}} - V_{\text{GA3P} \leftarrow \text{13BPG}} \frac{C_{\text{13BPG}} C_{\text{NADH}} C_{\text{H}^+}}{K_{\text{GA3P} \leftarrow \text{13BPG}}}}{1 + \frac{C_{\text{GA3P}} C_{\text{Pi}} C_{\text{NAD}^+}}{K_{\text{GA3P} \rightarrow \text{13BPG}}} + \frac{C_{\text{13BPG}} C_{\text{NADH}} C_{\text{H}^+}}{K_{\text{GA3P} \leftarrow \text{13BPG}}}} \right]$$

8. Phosphoglycerate Kinase



$$\phi_{\text{13BPG} \leftrightarrow \text{PEP}} = \left[\frac{V_{\text{13BPG} \rightarrow \text{PEP}} \frac{C_{\text{13BPG}} C_{\text{ADP}}}{K_{\text{13BPG} \rightarrow \text{PEP}}} - V_{\text{13BPG} \leftarrow \text{PEP}} \frac{C_{\text{PEP}} C_{\text{ATP}}}{K_{\text{13BPG} \leftarrow \text{PEP}}}}{1 + \frac{C_{\text{13BPG}} C_{\text{ADP}}}{K_{\text{13BPG} \rightarrow \text{PEP}}} + \frac{C_{\text{PEP}} C_{\text{ATP}}}{K_{\text{13BPG} \leftarrow \text{PEP}}}} \right]$$

This is a sum of 3 enzymatic reactions $\text{13BPG} + \text{ADP} \leftrightarrow \text{3PG} + \text{ATP}$, $\text{3PG} \leftrightarrow \text{2PG}$ and $\text{2PG} \leftrightarrow \text{PEP}$ catalyzed by the enzymes *phosphoglycerate kinase*, *phosphoglycerate mutase* and *enolase* (see Ref. (7), Ch. 15; Ref. (10), Ch. 19). Because these reactions are almost in equilibrium, they are combined to one reaction.

9. Pyruvate Kinase



$$\phi_{\text{PEP} \leftrightarrow \text{Pyr}} = \left[\frac{C_{\text{F16BP}}}{K_{\text{PEP} \leftrightarrow \text{PYR}}^{\text{Ctrl}} + C_{\text{F16BP}}} \right] \left[\frac{V_{\text{PEP} \rightarrow \text{Pyr}} \frac{C_{\text{PEP}} C_{\text{ADP}} C_{\text{H}^+}}{K_{\text{PEP} \rightarrow \text{Pyr}}} - V_{\text{PEP} \leftarrow \text{Pyr}} \frac{C_{\text{Pyr}} C_{\text{ATP}}}{K_{\text{PEP} \leftarrow \text{Pyr}}}}{1 + \frac{C_{\text{PEP}} C_{\text{ADP}} C_{\text{H}^+}}{K_{\text{PEP} \rightarrow \text{Pyr}}} + \frac{C_{\text{Pyr}} C_{\text{ATP}}}{K_{\text{PEP} \leftarrow \text{Pyr}}}} \right]$$

Pyruvate kinase is another key regulatory enzyme in the glycolytic pathway. This enzyme is known to be allosterically activated by F16BP (see Ref. (7), Ch. 15; Ref. (10), Ch. 19). Pyruvate is subsequently transported into the mitochondrial matrix where it is oxidized to produce ACoA.

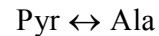
10. Lactate Dehydrogenase



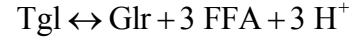
$$\phi_{\text{Pyr} \leftrightarrow \text{Lac}} = \left[\frac{V_{\text{Pyr} \rightarrow \text{Lac}} \frac{C_{\text{Pyr}} C_{\text{NADH}} C_{\text{H}^+}}{K_{\text{Pyr} \rightarrow \text{Lac}}} - V_{\text{Pyr} \leftarrow \text{Lac}} \frac{C_{\text{Lac}} C_{\text{NAD}^+}}{K_{\text{Pyr} \leftarrow \text{Lac}}}}{1 + \frac{C_{\text{Pyr}} C_{\text{NADH}} C_{\text{H}^+}}{K_{\text{Pyr} \rightarrow \text{Lac}}} + \frac{C_{\text{Lac}} C_{\text{NAD}^+}}{K_{\text{Pyr} \leftarrow \text{Lac}}}} \right]$$

This is an important reaction in skeletal muscle cellular metabolism and energetics. When the oxygen availability is limited (e.g., during muscle ischemia or intense muscle activities), NADH in cytosol can accumulate and reduce pyruvate to lactate with the help of the enzyme *lactate dehydrogenase* (see Ref. (7), Ch. 15; Ref. (10), Ch. 19).

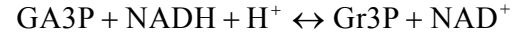
11. Alanine Formation (*Alanine Aminotransferase*)



$$\phi_{\text{Pyr} \leftrightarrow \text{Ala}} = \left[\frac{V_{\text{Pyr} \rightarrow \text{Ala}} \frac{C_{\text{Pyr}}}{K_{\text{Pyr} \rightarrow \text{Ala}}} - V_{\text{Pyr} \leftarrow \text{Ala}} \frac{C_{\text{Ala}}}{K_{\text{Pyr} \leftarrow \text{Ala}}}}{1 + \frac{C_{\text{Pyr}}}{K_{\text{Pyr} \rightarrow \text{Ala}}} + \frac{C_{\text{Ala}}}{K_{\text{Pyr} \leftarrow \text{Ala}}}} \right]$$

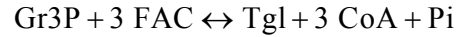
12. *Lipases (Triglycerides Hydrolysis)*


$$\phi_{\text{Tgl} \leftrightarrow \text{Glr}} = \left[\frac{V_{\text{Tgl} \rightarrow \text{Glr}} \frac{C_{\text{Tgl}}}{K_{\text{Tgl} \rightarrow \text{Glr}}} - V_{\text{Tgl} \leftarrow \text{Glr}} \frac{C_{\text{Glr}} C_{\text{FFA}}^3 C_{\text{H}^+}^3}{K_{\text{Tgl} \leftarrow \text{Glr}}}}{1 + \frac{C_{\text{Tgl}}}{K_{\text{Tgl} \rightarrow \text{Glr}}} + \frac{C_{\text{Glr}} C_{\text{FFA}}^3 C_{\text{H}^+}^3}{K_{\text{Tgl} \leftarrow \text{Glr}}}} \right]$$

13. *Glycerol 3-Phosphate Dehydrogenase*


$$\phi_{\text{GA3P} \leftrightarrow \text{Gr3P}} = \left[\frac{V_{\text{GA3P} \rightarrow \text{Gr3P}} \frac{C_{\text{GA3P}} C_{\text{NADH}} C_{\text{H}^+}}{K_{\text{GA3P} \rightarrow \text{Gr3P}}} - V_{\text{GA3P} \leftarrow \text{Gr3P}} \frac{C_{\text{Gr3P}} C_{\text{NAD}^+}}{K_{\text{GA3P} \leftarrow \text{Gr3P}}}}{1 + \frac{C_{\text{GA3P}} C_{\text{NADH}} C_{\text{H}^+}}{K_{\text{GA3P} \rightarrow \text{Gr3P}}} + \frac{C_{\text{Gr3P}} C_{\text{NAD}^+}}{K_{\text{GA3P} \leftarrow \text{Gr3P}}}} \right]$$

This reaction serves as the connection between glycolysis and lipid metabolism pathways. GA3P is ultimately utilized in the synthesis of triglycerides (see Ref. (7), Ch. 21).

14. *Acyltransferase (Triglyceride Synthesis)*


$$\phi_{\text{Gr3P} \leftrightarrow \text{Tgl}} = \left[\frac{V_{\text{Gr3P} \rightarrow \text{Tgl}} \frac{C_{\text{Gr3P}} C_{\text{FAC}}^3}{K_{\text{Gr3P} \rightarrow \text{Tgl}}} - V_{\text{Gr3P} \leftarrow \text{Tgl}} \frac{C_{\text{Tgl}} C_{\text{CoA}}^3 C_{\text{Pi}}}{K_{\text{Gr3P} \leftarrow \text{Tgl}}}}{1 + \frac{C_{\text{Gr3P}} C_{\text{FAC}}^3}{K_{\text{Gr3P} \rightarrow \text{Tgl}}} + \frac{C_{\text{Tgl}} C_{\text{CoA}}^3 C_{\text{Pi}}}{K_{\text{Gr3P} \leftarrow \text{Tgl}}}} \right]$$

This is a sum of 4 enzymatic reactions $\text{Gr3P} + \text{FAC} \leftrightarrow \text{LPPA} + \text{CoA}$, $\text{LPPA} + \text{FAC} \leftrightarrow \text{PPA} + \text{CoA}$, $\text{PPA} \leftrightarrow \text{DAG} + \text{Pi}$, $\text{DAG} + \text{FAC} \leftrightarrow \text{Tgl} + \text{CoA}$ catalyzed by the enzymes *Gr3P acyltransferase*, *LPPA acyltransferase*, *PPA phosphatase*, *DAG acyltransferase* (see Ref. (7), Ch. 21). The synthesis of triglycerides from glycerol is neglected in this model as the activity of the enzyme *glycerol kinase* is negligible in skeletal muscle.

15. *Acyl-CoA Synthetase*


$$\phi_{\text{FFA} \leftrightarrow \text{FAC}} = \left[\frac{V_{\text{FFA} \rightarrow \text{FAC}} \frac{C_{\text{FFA}} C_{\text{CoA}} C_{\text{ATP}}^2}{K_{\text{FFA} \rightarrow \text{FAC}}} - V_{\text{FFA} \leftarrow \text{FAC}} \frac{C_{\text{FAC}} C_{\text{ADP}}^2 C_{\text{Pi}}^2 C_{\text{H}^+}^2}{K_{\text{FFA} \leftarrow \text{FAC}}}}{1 + \frac{C_{\text{FFA}} C_{\text{CoA}} C_{\text{ATP}}^2}{K_{\text{FFA} \rightarrow \text{FAC}}} + \frac{C_{\text{FAC}} C_{\text{ADP}}^2 C_{\text{Pi}}^2 C_{\text{H}^+}^2}{K_{\text{FFA} \leftarrow \text{FAC}}}} \right]$$

This reaction is also called as *fatty acid activation* and the enzyme as *fatty acid thiokinase*. The activated fatty acid is transported into the mitochondrial matrix through carnitine shuttle which is subsequently oxidized into acetyl-CoA through several enzymatic reactions (see Ref. (7), Ch. 21; Ref. (10), Ch. 24).

16. *ATPase (ATP Hydrolysis)*


$$\phi_{\text{ATP} \leftrightarrow \text{ADP}} = \left[\frac{V_{\text{ATP} \rightarrow \text{ADP}} \frac{C_{\text{ATP}}}{K_{\text{ATP} \rightarrow \text{ADP}}} - V_{\text{ATP} \leftarrow \text{ADP}} \frac{C_{\text{ADP}} C_{\text{Pi}} C_{\text{H}^+}}{K_{\text{ATP} \leftarrow \text{ADP}}}}{1 + \frac{C_{\text{ATP}}}{K_{\text{ATP} \rightarrow \text{ADP}}} + \frac{C_{\text{ADP}} C_{\text{Pi}} C_{\text{H}^+}}{K_{\text{ATP} \leftarrow \text{ADP}}}} \right]$$

This reaction is the primary source of energy supply for muscle contraction (see Ref. (7), Ch. 14; Ref. (10), Ch. 17). It is known to be activated by Ca^{2+} which is not explicitly considered in this model of muscle metabolism.

17. Creatine Kinase



$$\phi_{\text{PCr} \leftrightarrow \text{Cr}} = \left[\frac{V_{\text{PCr} \rightarrow \text{Cr}} \frac{C_{\text{PCr}} C_{\text{ADP}} C_{\text{H}^+}}{K_{\text{PCr} \rightarrow \text{Cr}}} - V_{\text{PCr} \leftarrow \text{Cr}} \frac{C_{\text{Cr}} C_{\text{ATP}}}{K_{\text{PCr} \leftarrow \text{Cr}}}}{1 + \frac{C_{\text{PCr}} C_{\text{ADP}} C_{\text{H}^+}}{K_{\text{PCr} \rightarrow \text{Cr}}} + \frac{C_{\text{Cr}} C_{\text{ATP}}}{K_{\text{PCr} \leftarrow \text{Cr}}}} \right]$$

This is a buffer reaction and functions to maintain ATP homeostasis during muscle contraction (see Ref. (7), Ch. 14; Ref. (10), Ch. 17). It is the source of immediate energy supply during the transitions from rest to exercise.

18. Adenylate Kinase



$$\phi_{\text{AMP} \leftrightarrow \text{ADP}} = \left[\frac{V_{\text{AMP} \rightarrow \text{ADP}} \frac{C_{\text{ATP}} C_{\text{AMP}}}{K_{\text{AMP} \rightarrow \text{ADP}}} - V_{\text{AMP} \leftarrow \text{ADP}} \frac{C_{\text{ADP}}^2}{K_{\text{AMP} \leftarrow \text{ADP}}}}{1 + \frac{C_{\text{ATP}} C_{\text{AMP}}}{K_{\text{AMP} \rightarrow \text{ADP}}} + \frac{C_{\text{ADP}}^2}{K_{\text{AMP} \leftarrow \text{ADP}}}} \right]$$

Reactions in Mitochondria

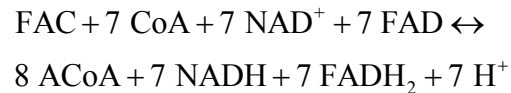
19. Pyruvate Dehydrogenase



$$\phi_{\text{Pyr} \leftrightarrow \text{ACoA}} = \left[\frac{\frac{C_{\text{ADP}}}{C_{\text{ATP}}}}{K_{\text{Pyr} \leftrightarrow \text{ACoA}}^{\text{Ctrl}} + \frac{C_{\text{ADP}}}{C_{\text{ATP}}}} \right] \left[\frac{V_{\text{Pyr} \rightarrow \text{ACoA}} \frac{C_{\text{Pyr}} C_{\text{CoA}} C_{\text{NAD}^+}}{K_{\text{Pyr} \rightarrow \text{ACoA}}} - V_{\text{Pyr} \leftarrow \text{ACoA}} \frac{C_{\text{ACoA}} C_{\text{CO}_2} C_{\text{NADH}} C_{\text{H}^+}}{K_{\text{Pyr} \leftarrow \text{ACoA}}}}{1 + \frac{C_{\text{Pyr}} C_{\text{CoA}} C_{\text{NAD}^+}}{K_{\text{Pyr} \rightarrow \text{ACoA}}} + \frac{C_{\text{ACoA}} C_{\text{CO}_2} C_{\text{NADH}} C_{\text{H}^+}}{K_{\text{Pyr} \leftarrow \text{ACoA}}}} \right]$$

This is the first reaction inside the mitochondrial matrix in which ACoA is formed from the oxidative decarboxylation of pyruvate leading to the TCA cycle (see Ref. (7), Ch. 16; Ref. (10), Ch. 20). The enzyme *pyruvate dehydrogenase* is known to be allosterically activated by ADP and inhibited by ATP. Thus this reaction is assumed to be controlled by the concentration ratio $C_{\text{ADP}}/C_{\text{ATP}}$. The enzyme is also known to be inhibited by ACoA and NADH which are included as product inhibitors. The enzyme is also known to be activated by Ca^{2+} which is not explicitly included in this model of muscle metabolism.

20. Fatty Acyl-CoA Oxidation (β -Oxidation)



$$\phi_{\text{FAC} \leftrightarrow \text{ACoA}} = \left[\frac{V_{\text{FAC} \rightarrow \text{ACoA}} \frac{C_{\text{FAC}} C_{\text{CoA}} C_{\text{NAD}^+} C_{\text{FAD}}}{K_{\text{FAC} \rightarrow \text{ACoA}}} - V_{\text{FAC} \leftarrow \text{ACoA}} \frac{C_{\text{ACoA}} C_{\text{NADH}} C_{\text{FADH}_2} C_{\text{H}^+}}{K_{\text{FAC} \leftarrow \text{ACoA}}}}{1 + \frac{C_{\text{FAC}} C_{\text{CoA}} C_{\text{NAD}^+} C_{\text{FAD}}}{K_{\text{FAC} \rightarrow \text{ACoA}}} + \frac{C_{\text{ACoA}} C_{\text{NADH}} C_{\text{FADH}_2} C_{\text{H}^+}}{K_{\text{FAC} \leftarrow \text{ACoA}}}} \right]$$

This reaction producing ACoA from the activated fatty acid inside the mitochondrial matrix is highly complex. It is the result of combining 7 cycles of reactions in which each cycle consists of 4 enzymatic reactions catalyzed by the enzymes *acyl-CoA dehydrogenase*, *enoyl-CoA hydratase*, *beta-hydroxyacyl-CoA dehydrogenase*, and *acyl-CoA acetyltransferase (thiolase)* (see Ref. (7), Ch. 21; Ref. (10), Ch. 24).

21. Citrate Synthase



$$\phi_{\text{ACoA} \leftrightarrow \text{Cit}} = \left[\frac{\frac{C_{\text{ADP}}}{C_{\text{ATP}}}}{K_{\text{ACoA} \leftrightarrow \text{Cit}}^{\text{Ctrl}} + \frac{C_{\text{ADP}}}{C_{\text{ATP}}}} \right] \left[\frac{V_{\text{ACoA} \rightarrow \text{Cit}} \frac{C_{\text{ACoA}} C_{\text{Oxa}}}{K_{\text{ACoA} \rightarrow \text{Cit}}} - V_{\text{ACoA} \leftarrow \text{Cit}} \frac{C_{\text{Cit}} C_{\text{CoA}} C_{\text{H}^+}}{K_{\text{ACoA} \leftarrow \text{Cit}}}}{1 + \frac{C_{\text{ACoA}} C_{\text{Oxa}}}{K_{\text{ACoA} \rightarrow \text{Cit}}} + \frac{C_{\text{Cit}} C_{\text{CoA}} C_{\text{H}^+}}{K_{\text{ACoA} \leftarrow \text{Cit}}}} \right]$$

This is the first reaction in TCA cycle. The enzyme *citrate synthase* is known to be allosterically activated by ADP and inhibited by ATP (see Ref. (7), Ch.16; Ref. (10), Ch.20). Thus the reaction is assumed to be controlled by the concentration ratio $C_{\text{ADP}}/C_{\text{ATP}}$. The enzyme is also known to be inhibited by SCoA which is included as a product inhibitor.

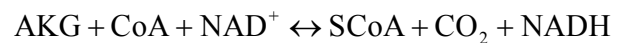
22. Aconitase + Isocitrate Dehydrogenase



$$\phi_{\text{Cit} \leftrightarrow \text{AKG}} = \left[\frac{\frac{C_{\text{ADP}}}{C_{\text{ATP}}}}{K_{\text{Cit} \leftrightarrow \text{AKG}}^{\text{Ctrl}} + \frac{C_{\text{ADP}}}{C_{\text{ATP}}}} \right] \left[\frac{V_{\text{Cit} \rightarrow \text{AKG}} \frac{C_{\text{Cit}} C_{\text{NAD}^+}}{K_{\text{Cit} \rightarrow \text{AKG}}} - V_{\text{Cit} \leftarrow \text{AKG}} \frac{C_{\text{AKG}} C_{\text{CO}_2} C_{\text{NADH}}}{K_{\text{Cit} \leftarrow \text{AKG}}}}{1 + \frac{C_{\text{Cit}} C_{\text{NAD}^+}}{K_{\text{Cit} \rightarrow \text{AKG}}} + \frac{C_{\text{AKG}} C_{\text{CO}_2} C_{\text{NADH}}}{K_{\text{Cit} \leftarrow \text{AKG}}}} \right]$$

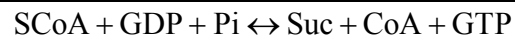
This is a sum of 2 enzymatic reactions $\text{Cit} \leftrightarrow \text{ICit}$ and $\text{ICit} + \text{NAD}^+ \leftrightarrow \text{AKG} + \text{CO}_2 + \text{NADH}$ catalyzed by the enzymes *aconitase* and *isocitrate dehydrogenase*. This reaction is known to be activated by ADP and inhibited by ATP (see Ref. (7), Ch. 16; Ref. (10), Ch. 20). Thus this reaction is considered to be controlled by the concentration ratio $C_{\text{ADP}}/C_{\text{ATP}}$. This reaction is also known to be activated by Ca^{2+} which is not explicitly included in this model of muscle metabolism.

23. α -Ketoglutarate Dehydrogenase



$$\phi_{\text{AKG} \leftrightarrow \text{SCoA}} = \left[\frac{V_{\text{AKG} \rightarrow \text{SCoA}} \frac{C_{\text{AKG}} C_{\text{CoA}} C_{\text{NAD}^+}}{K_{\text{AKG} \rightarrow \text{SCoA}}} - V_{\text{AKG} \leftarrow \text{SCoA}} \frac{C_{\text{SCoA}} C_{\text{CO}_2} C_{\text{NADH}}}{K_{\text{AKG} \leftarrow \text{SCoA}}}}{1 + \frac{C_{\text{AKG}} C_{\text{CoA}} C_{\text{NAD}^+}}{K_{\text{AKG} \rightarrow \text{SCoA}}} + \frac{C_{\text{SCoA}} C_{\text{CO}_2} C_{\text{NADH}}}{K_{\text{AKG} \leftarrow \text{SCoA}}}} \right]$$

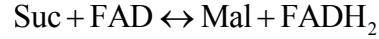
24. Succinyl-CoA Synthetase



$$\phi_{\text{SCoA} \leftrightarrow \text{Suc}} = \left[\frac{V_{\text{SCoA} \rightarrow \text{Suc}} \frac{C_{\text{SCoA}} C_{\text{ADP}} C_{\text{Pi}}}{K_{\text{SCoA} \rightarrow \text{Suc}}} - V_{\text{SCoA} \leftarrow \text{Suc}} \frac{C_{\text{Suc}} C_{\text{CoA}} C_{\text{ATP}}}{K_{\text{SCoA} \leftarrow \text{Suc}}}}{1 + \frac{C_{\text{SCoA}} C_{\text{ADP}} C_{\text{Pi}}}{K_{\text{SCoA} \rightarrow \text{Suc}}} + \frac{C_{\text{Suc}} C_{\text{CoA}} C_{\text{ATP}}}{K_{\text{SCoA} \leftarrow \text{Suc}}}} \right]$$

Because the reaction $\text{GTP} + \text{ADP} \leftrightarrow \text{GDP} + \text{ATP}$ is in fast equilibrium, we assume the GTP/GDP ratio to be proportional to the ATP/ADP ratio (see Ref. (7), Ch. 16; Ref. (10), Ch. 20).

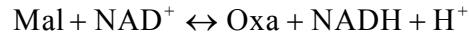
25. Succinate Dehydrogenase



$$\phi_{\text{Suc} \leftrightarrow \text{Mal}} = \left[\frac{V_{\text{Suc} \rightarrow \text{Mal}} \frac{C_{\text{Suc}} C_{\text{FAD}}}{K_{\text{Suc} \rightarrow \text{Mal}}} - V_{\text{Suc} \leftarrow \text{Mal}} \frac{C_{\text{Mal}} C_{\text{FADH}_2}}{K_{\text{Suc} \leftarrow \text{Mal}}}}{1 + \frac{C_{\text{Suc}} C_{\text{FAD}}}{K_{\text{Suc} \rightarrow \text{Mal}}} + \frac{C_{\text{Mal}} C_{\text{FADH}_2}}{K_{\text{Suc} \leftarrow \text{Mal}}}} \right]$$

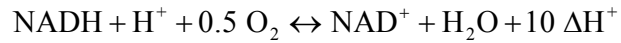
This is a sum of two enzymatic reactions $\text{Suc} + \text{FAD} \leftrightarrow \text{Fum} + \text{FADH}_2$ and $\text{Fum} \leftrightarrow \text{Mal}$ catalyzed by the enzymes *succinate dehydrogenase* and *fumarate* (see Ref. (7), Ch. 16; Ref. (10), Ch. 20).

26. Malate Dehydrogenase



$$\phi_{\text{Mal} \leftrightarrow \text{Oxa}} = \left[\frac{V_{\text{Mal} \rightarrow \text{Oxa}} \frac{C_{\text{Mal}} C_{\text{NAD}^+}}{K_{\text{Mal} \rightarrow \text{Oxa}}} - V_{\text{Mal} \leftarrow \text{Oxa}} \frac{C_{\text{Oxa}} C_{\text{NADH}} C_{\text{H}^+}}{K_{\text{Mal} \leftarrow \text{Oxa}}}}{1 + \frac{C_{\text{Mal}} C_{\text{NAD}^+}}{K_{\text{Mal} \rightarrow \text{Oxa}}} + \frac{C_{\text{Oxa}} C_{\text{NADH}} C_{\text{H}^+}}{K_{\text{Mal} \leftarrow \text{Oxa}}}} \right]$$

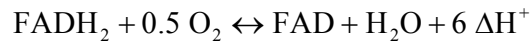
27. Complex I+III+IV



$$\phi_{\text{O}_2 \leftrightarrow \text{H}_2\text{O}, \text{NADH}} = \left[\frac{\exp\left(-\frac{10\Delta G_{\text{H}^+}}{RT}\right) V_{\text{O}_2 \rightarrow \text{H}_2\text{O}, \text{NADH}} \frac{C_{\text{NADH}} C_{\text{O}_2}^{0.5} C_{\text{H}^+}}{K_{\text{O}_2 \rightarrow \text{H}_2\text{O}, \text{NADH}}} - V_{\text{O}_2 \leftarrow \text{H}_2\text{O}, \text{NADH}} \frac{C_{\text{NAD}^+}}{K_{\text{O}_2 \leftarrow \text{H}_2\text{O}, \text{NADH}}}}{1 + \frac{C_{\text{NADH}} C_{\text{O}_2}^{0.5} C_{\text{H}^+}}{K_{\text{O}_2 \rightarrow \text{H}_2\text{O}, \text{NADH}}} + \frac{C_{\text{NAD}^+}}{K_{\text{O}_2 \leftarrow \text{H}_2\text{O}, \text{NADH}}}} \right]$$

This lumped reaction in the electron transport chain involving NADH-NAD⁺ pairs requires pumping of 10 protons from the mitochondrial matrix into the inner membrane space (see Ref. (7), Ch. 19; Ref. (10), Ch. 21). Thus the flux of the forward reaction is modified here to depend on the proton motive force defined by $\Delta G_{\text{H}^+} = F\Delta\Psi + RT \ln(C_{\text{H}^+_{\text{cyt}}} / C_{\text{H}^+_{\text{mit}}})$ where F is the Faraday's constant and $\Delta\Psi$ is the mitochondrial membrane potential.

28. Complex II+III+IV



$$\phi_{\text{O}_2 \leftrightarrow \text{H}_2\text{O}, \text{FADH}_2} = \left[\frac{\exp\left(-\frac{6\Delta G_{\text{H}^+}}{RT}\right) V_{\text{O}_2 \rightarrow \text{H}_2\text{O}, \text{FADH}_2} \frac{C_{\text{FADH}_2} C_{\text{O}_2}^{0.5}}{K_{\text{O}_2 \rightarrow \text{H}_2\text{O}, \text{FADH}_2}} - V_{\text{O}_2 \leftarrow \text{H}_2\text{O}, \text{FADH}_2} \frac{C_{\text{FAD}}}{K_{\text{O}_2 \leftarrow \text{H}_2\text{O}, \text{FADH}_2}}}{1 + \frac{C_{\text{FADH}_2} C_{\text{O}_2}^{0.5}}{K_{\text{O}_2 \rightarrow \text{H}_2\text{O}, \text{FADH}_2}} + \frac{C_{\text{FAD}}}{K_{\text{O}_2 \leftarrow \text{H}_2\text{O}, \text{FADH}_2}} \right]$$

This lumped reaction in the electron transport chain involving FADH₂-FAD pairs requires pump-

ing of 6 protons from the mitochondrial matrix into the inner membrane space (see Ref. (7), Ch. 19; Ref. (10), Ch. 21). The forward reaction flux is modified here to depend on the proton motive force ΔG_{H^+} .

29. *ATP Synthesis (F₁F₀-ATPase or Complex V)* ADP + Pi + H⁺ + 3ΔH⁺ ↔ ATP

$$\phi_{ADP \leftrightarrow ATP} = \left[\frac{\exp\left(+\frac{3\Delta G_{H^+}}{RT}\right) V_{ADP \rightarrow ATP} \frac{C_{ADP} C_{Pi} C_{H^+}}{K_{ADP \rightarrow ATP}} - V_{ADP \leftarrow ATP} \frac{C_{ATP}}{K_{ADP \leftarrow ATP}}}{1 + \frac{C_{ADP} C_{Pi} C_{H^+}}{K_{ADP \rightarrow ATP}} + \frac{C_{ATP}}{K_{ADP \leftarrow ATP}}} \right]$$

ADP is phosphorylated to ATP inside the mitochondrial matrix via the F₁F₀-ATPase (Complex V) reaction (see Ref. (7), Ch. 19; Ref. (10), Ch. 21). This requires 3 protons to be pumped out from the inner membrane space into the mitochondrial matrix. The forward reaction flux is modified here to depend on the proton motive force ΔG_{H^+} .

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