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

Yanjun Li[1,2], Ranjan K. Dash[5,6], Jaeyeon Kim[1,2], Gerald M. Saidel[1,2], and Marco E. Cabrera[1,2,3,4]

Center for Modeling Integrated Metabolic Systems[1] and Departments of Biomedical Engineering[2], Physiology and Biophysics[3], and Pediatrics[4], Case Western Reserve University, Cleveland, OH - 44106

Biotechnology and Bioengineering Center[5] and Department of Physiology[6], Medical College of Wisconsin, Milwaukee, WI - 53226

Address for correspondence: Marco E. Cabrera, Ph.D., Pediatric Cardiology, RBC-389, MS 6011, Case Western Reserve University, 11100 Euclid Avenue, Cleveland, OH 44106-6011. Phone: (216) 844-5085, Fax: (216) 844-5478, Email: mec6@cwru.edu

APPENDIX A: DYNAMIC MASS BALANCE EQUATIONS OF O_2 AND CO_2

O_2 transport dynamics in skeletal muscle

In RBCs, O_2 is transported as free dissolved O_2 and as bound oxy-hemoglobin (HbO_2), while in mitochondria, O_2 is transported as dissolved O_2 and as oxy-myoglobin (MbO_2). In contrast, in plasma, ISF and cytosol, O_2 is transported only as dissolved O_2. Therefore, the dynamic mass balance equations of O_2 must account for different forms of O_2 transport in RBCs, plasma, ISF, cytosol and mitochondria (2; 5; 6).

The assumptions of perfect mixing in each phase and phase equilibrium between free O_2 in plasma, RBC and ISF yields $C^F_{bl,O2} = C^F_{pl,O2} = C^F_{rbc,O2} = C^F_{isf,O2}$. Furthermore, by assuming the on and off binding rates of O_2 to hemoglobin in RBCs and O_2 to myoglobin in mitochondria are fast compared to the transport processes, the detailed kinetics of the binding can be neglected and the free dissolved O_2 can be considered to be in instantaneous equilibrium with the HbO_2 in RBCs and MbO_2 in mitochondria (2).

The combined dynamic mass balance equations for O_2 in capillary blood domain in equilibrium with tissue ISF domain can be written as

$$V_{bl}\frac{dC^T_{bl,O2}}{dt} + V_{isf}\frac{dC^F_{isf,O2}}{dt} = Q\left(C^T_{art,O2} - C^T_{bl,O2}\right) - J^p_{bl\leftrightarrow cyt,O2} \qquad \text{(A1-a)}$$

and the compartmentalized dynamic mass balance equations for O_2 in tissue subcellular (cytosolic and mitochondrial) domains can be written as

$$V_{cyt}\frac{dC^{F}_{cyt,O2}}{dt}=J^{p}_{bl\leftrightarrow cyt,O2}-J^{p}_{cyt\leftrightarrow mit,O2} \tag{A1-b}$$

$$V_{mit}\frac{dC^{T}_{mit,O2}}{dt}=J^{p}_{cyt\leftrightarrow mit,O2}-0.5\phi_{O2\leftrightarrow H2O,NADH}-0.5\phi_{O2\leftrightarrow H2O,FADH2} \tag{A1-c}$$

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

$$C^{T}_{x,O2}=C^{F}_{x,O2}+C_{x,HbO2},\quad x=(art,bl) \tag{A2-a}$$

and in tissue cells mitochondria, the concentrations are related by

$$C^{T}_{mit,O2}=C^{F}_{mit,O2}+C_{mit,MbO2} \tag{A2-b}$$

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

$$J^{p}_{bl\leftrightarrow cyt,O2}=\lambda_{bl\leftrightarrow cyt,O2}\left(C^{F}_{bl,O2}-C^{F}_{cyt,O2}\right) \tag{A3-a}$$

$$J^{p}_{cyt\leftrightarrow mit,O2}=\lambda_{cyt\leftrightarrow mit,O2}\left(C^{F}_{cyt,O2}-C^{F}_{mit,O2}\right) \tag{A3-b}$$

The concentrations of HbO_2 and MbO_2 can be written in terms of their saturations (1; 2) as

$$C_{x,HbO2}=4.H_{rbc}.C_{rbc,Hb}.S_{x,HbO2},\quad S_{x,HbO2}=\frac{K_{HbO2}.\left(C^{F}_{x,O2}\right)^{n_H}}{1+K_{HbO2}.\left(C^{F}_{x,O2}\right)^{n_H}} \tag{A4-a}$$

$$C_{mit,MbO2}=C_{mit,Mb}.S_{mit,MbO2},\quad S_{mit,MbO2}=\frac{K_{MbO2}.C^{F}_{mit,O2}}{1+K_{MbO2}.C^{F}_{mit,O2}} \tag{A4-b}$$

Substituting Eq. (A4) in Eq. (A2), we have the expressions for total O_2 concentrations as

$$C^{T}_{x,O2}=C^{F}_{x,O2}+\frac{4.H_{rbc}.C_{rbc,Hb}.K_{HbO2}.\left(C^{F}_{x,O2}\right)^{n_H}}{1+K_{HbO2}.\left(C^{F}_{x,O2}\right)^{n_H}},\quad x=(art,bl) \tag{A5-a}$$

$$C^{T}_{mit,O2}=C^{F}_{mit,O2}+\frac{C_{mit,Mb}.K_{MbO2}.C^{F}_{mit,O2}}{1+K_{MbO2}.C^{F}_{mit,O2}} \tag{A5-b}$$

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

$$\left(V_{bl,O2}+V_{isf,O2}\right)\frac{dC^{F}_{bl,O2}}{dt}=Q\left(C^{T}_{art,O2}-C^{T}_{bl,O2}\right)-J^{p}_{bl\leftrightarrow cyt,O2} \tag{A6-a}$$

$$V_{cyt,O2}\frac{dC^{F}_{cyt,O2}}{dt}=J^{p}_{bl\leftrightarrow cyt,O2}-J^{p}_{cyt\leftrightarrow mit,O2} \tag{A6-b}$$

$$V_{mit,O2}\frac{dC^{F}_{mit,O2}}{dt} = J^{p}_{cyt\leftrightarrow mit,O2} - 0.5\phi_{O2\leftrightarrow H2O,NADH} - 0.5\phi_{O2\leftrightarrow H2O,FADH2} \quad \text{(A6-c)}$$

where the effective volumes or volumes of distributions of O_2 in capillary blood, tissue ISF, and tissue subcellular domains (cytosol and mitochondria) ($V_{bl,O2}$, $V_{isf,O2}$, $V_{cyt,O2}$, $V_{mit,O2}$) are given by

$$V_{bl,O2} = V_{bl}\left(1+\frac{4.n_H.H_{rbc}.C_{rbc,Hb}.K_{HbO2}.\left(C^{F}_{bl,O2}\right)^{n_H-1}}{\left[1+K_{HbO2}.\left(C^{F}_{bl,O2}\right)^{n_H}\right]^{2}}\right), \quad V_{isf,O2} = V_{isf} \quad \text{(A7-a)}$$

$$V_{cyt,O2} = V_{cyt}, \quad V_{mit,O2} = V_{mit}\left(1+\frac{C_{mit,Mb}.K_{MbO2}}{\left[1+K_{MbO2}.C^{F}_{mit,O2}\right]^{2}}\right) \quad \text{(A7-b)}$$

CO_2 transport dynamics in skeletal muscle

In RBCs, CO_2 is transported as free dissolved CO_2, as bound carbamino-hemoglobin ($HbCO_2$), and as bicarbonate (HCO_3^-), while in plasma, ISF, cytosol and mitochondria, CO_2 is transported only as dissolved CO_2 and as HCO_3^-. Therefore, the dynamic mass balance equations for CO_2 must account for different forms of CO_2 transport in RBCs, plasma, ISF, cytosol and mitochondria (2; 4).

The assumptions of perfect mixing in each phase and phase equilibrium between free CO_2 in plasma, RBC and ISF yields $C^{F}_{bl,CO2} = C^{F}_{pl,CO2} = C^{F}_{rbc,CO2} = C^{F}_{isf,CO2}$. Furthermore, by assuming that the on and off binding rate of CO_2 to hemoglobin in RBCs is fast compared to the transport processes, the detailed kinetics of the binding can be neglected and the dissolved CO_2 can be considered to be in instantaneous equilibrium with the $HbCO_2$ in blood (2; 4).

The combined dynamic mass balance equation for CO_2 in capillary blood domain in equilibrium with tissue ISF domain can be written as

$$V_{bl}\frac{dC^{T}_{bl,CO2}}{dt} + V_{isf}\frac{dC^{T}_{isf,CO2}}{dt} = Q\left(C^{T}_{art,CO2} - C^{T}_{bl,CO2}\right) - J^{p}_{bl\leftrightarrow cyt,CO2} \quad \text{(A8-a)}$$

and the compartmentalized dynamic mass balance equations for CO_2 in tissue subcellular (cytosolic and mitochondrial) domains can be written as

$$V_{cyt}\frac{dC^{T}_{cyt,CO2}}{dt} = J^{p}_{bl\leftrightarrow cyt,CO2} - J^{p}_{cyt\leftrightarrow mit,CO2} \quad \text{(A8-b)}$$

$$V_{mit}\frac{dC^{T}_{mit,CO2}}{dt} = J^{p}_{cyt\leftrightarrow mit,CO2} + \left(\phi_{PYR\leftrightarrow ACoA} + \phi_{CIT\leftrightarrow AKG} + \phi_{AKG\leftrightarrow SCoA}\right) \quad \text{(A8-c)}$$

where

$$C^T_{x,CO2} = C^F_{x,CO2} + C_{x,HbCO2} + C_{x,HCO3\text{-}}, \quad x = (art, bl) \tag{A9-a}$$

$$C^T_{x,CO2} = C^F_{x,CO2} + C_{x,HCO3\text{-}}, \quad x = (isf, cyt, mit) \tag{A9-b}$$

$$J^p_{bl\leftrightarrow cyt,CO2} = \lambda_{bl\leftrightarrow cyt,CO2}\left(C^F_{bl,CO2} - C^F_{cyt,CO2}\right) \tag{A10-a}$$

$$J^p_{cyt\leftrightarrow mit,CO2} = \lambda_{cyt\leftrightarrow mit,CO2}\left(C^F_{cyt,CO2} - C^F_{mit,CO2}\right) \tag{A10-b}$$

The concentration of $HbCO_2$ can be written in terms of its saturation (1; 2) as

$$C_{x,HbCO2} = 4.H_{rbc}.C_{rbc,Hb}.S_{x,HbCO2},\ S_{x,HbCO2} = \frac{K_{HbCO2}.C^F_{x,CO2}}{1 + K_{HbCO2}.C^F_{x,CO2}},\ x = (art, bl) \tag{A11-a}$$

Applying the Henderson-Hasselbalch relation (1; 2; 4), we obtain the concentrations of HCO_3^- in the intravascular phases as

$$C_{x,HCO3\text{-}} = \left[\frac{(1 - H_{rbc})}{C_{x,pl,H+}} + \frac{H_{rbc}}{C_{x,rbc,H+}}\right] K_{CO2hyd}.C^F_{x,CO2}, \quad x = (art, bl) \tag{A11-b}$$

and the concentrations of HCO_3^- in the extravascular phases as

$$C_{x,HCO3\text{-}} = \frac{K_{CO2hyd}.C^F_{x,CO2}}{C_{x,H+}}, \quad x = (isf, cyt, mit) \tag{A11-c}$$

Substituting Eq. (A11) in Eq. (A9), we have the expressions for total CO_2 concentrations as

$$\begin{aligned} C^T_{x,CO2} &= C^F_{x,CO2} + \frac{4.H_{rbc}.C_{rbc,Hb}.K_{HbCO2}.C^F_{x,CO2}}{1 + K_{HbCO2}.C^F_{x,CO2}} \\ &+ \left[\frac{(1 - H_{rbc})}{C_{x,pl,H+}} + \frac{H_{rbc}}{C_{x,rbc,H+}}\right] K_{CO2hyd}.C^F_{x,CO2}, \quad x = (art, bl) \end{aligned} \tag{A12-a}$$

$$C^T_{x,CO2} = C^F_{x,CO2} + \frac{K_{CO2hyd}.C^F_{x,CO2}}{C_{x,H+}}, \quad x = (isf, cyt, mit) \tag{A12-b}$$

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

$$\left(V_{bl,CO2} + V_{isf,CO2}\right)\frac{dC^F_{bl,CO2}}{dt} = Q\left(C^T_{art,CO2} - C^T_{bl,CO2}\right) - J^p_{bl\leftrightarrow cyt,CO2} \tag{A13-a}$$

$$V_{cyt,CO2}\frac{dC^F_{cyt,CO2}}{dt} = J^p_{bl\leftrightarrow cyt,CO2} - J^p_{cyt\leftrightarrow mit,CO2} \tag{A13-b}$$

$$V_{mit,CO2}\frac{dC^{F}_{mit,CO2}}{dt} = J^{p}_{cyt\leftrightarrow mit,CO2} + \left(\phi_{PYR\leftrightarrow ACoA} + \phi_{CIT\leftrightarrow AKG} + \phi_{AKG\leftrightarrow SCoA}\right) \qquad \text{(A13-c)}$$

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

$$V_{bl,CO2} = V_{bl}\left(1 + \frac{4.H_{rbc}.C_{rbc,Hb}.K_{HbCO2}}{\left[1+K_{HbCO2}.C^{F}_{bl,CO2}\right]^{2}} + \left[\frac{(1-H_{rbc})}{C_{bl,pl,H+}} + \frac{H_{rbc}}{C_{bl,rbc,H+}}\right]K_{CO2hyd}\right) \qquad \text{(A14-a)}$$

$$V_{x,CO2} = V_{x}\left(1 + \frac{K_{CO2hyd}}{C_{x,H^{+}}}\right), \quad x = (isf, cyt, mit) \qquad \text{(A14-b)}$$

Model parameters: In the dynamics mass balance equations for O_2 and CO_2, $C_{rbc,Hb}$ = 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 HbO_2 saturation, K_{HbO2} = 7800.7 $mM^{-2.7}$ is the Hill constant for HbO_2 saturation, $C_{cl,Mb} = H_{mit}.C_{mit,Mb}$ = 0.5 mM is the concentration of myoglobin in tissue cells, H_{mit} = 0.1 is the fraction of mitochondria in tissue cells, K_{MbO2} = 308.64 mM^{-1} is the Hill constant for MbO_2 saturation, K_{HbCO2} = 0.1237 mM^{-1} is the Hill constant for $HbCO_2$ saturation, and K_{CO2hyd} = 7.95E-04 mM is the equilibrium constant for CO_2 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. $$\left(V_{bl}+V_{isf}\right)\frac{dC_{bl,Glc}}{dt} = Q\left(C_{art,Glc} - C_{bl,Glc}\right) - J^{f}_{bl\leftrightarrow cyt,Glc}$$

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

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

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

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

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

B7. $$\left(V_{bl,CO2}+V_{isf,CO2}\right)\frac{dC^{F}_{bl,CO2}}{dt}=Q\left(C^{T}_{art,CO2}-C^{T}_{bl,CO2}\right)-J^{p}_{bl\leftrightarrow cyt,CO2}$$

B8. $$\left(V_{bl,O2}+V_{isf,O2}\right)\frac{dC^{F}_{bl,O2}}{dt}=Q\left(C^{T}_{art,O2}-C^{T}_{bl,O2}\right)-J^{p}_{bl\leftrightarrow cyt,O2}$$

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

Tissue Cells Cytosolic Domain:

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

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

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

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

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

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

C7. $$V_{cyt,CO2}\frac{dC^{F}_{cyt,CO2}}{dt}=J^{p}_{bl\leftrightarrow cyt,CO2}-J^{p}_{cyt\leftrightarrow mit,CO2}$$

C8. $$V_{cyt,O2}\frac{dC^{F}_{cyt,O2}}{dt}=J^{p}_{bl\leftrightarrow cyt,O2}-J^{p}_{cyt\leftrightarrow mit,O2}$$

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

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

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

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

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

C20. $$V_{cyt}\frac{dC_{cyt,ATP}}{dt}=\begin{pmatrix}-J^{f}_{cyt\leftrightarrow mit,ATP/ADP}-\phi_{G6P\leftrightarrow Gly}-\phi_{Glc\leftrightarrow G6P}-\phi_{F6P\leftrightarrow F16BP}+\phi_{13BPG\leftrightarrow PEP}\\+\phi_{PEP\leftrightarrow Pyr}-2\phi_{FFA\leftrightarrow FAC}-\phi_{ATP\leftrightarrow ADP}+\phi_{PCr\leftrightarrow Cr}-\phi_{AMP\leftrightarrow ADP}\end{pmatrix}=-V_{cyt}\frac{dC_{cyt,ADP}}{dt}$$

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

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

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

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

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

where β_{cyt} = 6.65 mM/pH is buffering capacity of cytosol for protons

Tissue Cells Mitochondrial Domain:

M1. $$V_{mit,O2}\frac{dC^{F}_{mit,O2}}{dt} = J^{p}_{cyt\leftrightarrow mit,O2} - 0.5\phi_{O2\leftrightarrow H2O,NADH} - 0.5\phi_{O2\leftrightarrow H2O,FADH2}$$

M2. $$V_{mit,CO2}\frac{dC^{F}_{mit,CO2}}{dt} = J^{p}_{cyt\leftrightarrow mit,CO2} + \left(\phi_{PYR\leftrightarrow ACoA} + \phi_{CIT\leftrightarrow AKG} + \phi_{AKG\leftrightarrow SCoA}\right)$$

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

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

M5. $$V_{mit}\frac{dC_{mit,NADH}}{dt} = \begin{pmatrix} J^{f}_{cyt\leftrightarrow mit,NADH/NAD^{+}} + \phi_{Pyr\leftrightarrow ACoA} + 7\phi_{FAC\leftrightarrow ACoA} + \phi_{Cit\leftrightarrow AKG} \\ + \phi_{AKG\leftrightarrow SCoA} + \phi_{Mal\leftrightarrow Oxa} - \phi_{O2\leftrightarrow H2O,NADH} \end{pmatrix} = -V_{mit}\frac{dC_{mit,NAD^{+}}}{dt}$$

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

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

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

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

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

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

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

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

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

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

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

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

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

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

where $C_{\text{IMM}} = 6.75*10^{-3}$ 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:

$$\phi^0_{mit,ADP\leftrightarrow ATP} = 2.5\phi^0_{O_2\leftrightarrow H_2O,NADH} + 1.5\phi^0_{O_2\leftrightarrow H_2O,FADH2} \qquad \text{(C1)}$$

The basis for this relationship is that in oxidative phosphorylation, 1 mole of NADH can produce 2.5 moles of ATP and 1 mole $FADH_2$ 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:

$$\phi^0_{PCr\leftrightarrow Cr} = 0 \text{ and } \phi^0_{AMP\leftrightarrow ADP} = 0 \qquad \text{(C2)}$$

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

$$f^0_{cyt,ATP\rightarrow ADP} = f^0_{13BPG\rightarrow PEP} + f^0_{PEP\rightarrow Pyr} - f^0_{Glc\rightarrow G6P} - f^0_{G6P\rightarrow Gly} - f^0_{F6P\rightarrow F16BP} - 2f^0_{FFA\rightarrow FAC} - J^{f,0}_{c\rightarrow m,ATP/ADP} \qquad \text{(C3)}$$

Metabolic Reactions	*Flux Relations Arranged for Sequential Solution*	*Mass Balance* (*Appendix B*)
Hexokinase	$f^0_{Glc\rightarrow G6P} = J^{f,0}_{b\rightarrow c,Glc}$	*C1*
Phosphoglucose isomerase	$f^0_{G6P\rightarrow F6P} = f^0_{Glc\rightarrow G6P}$	*C10*

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

ATPase (ATP synthesis)	$\phi^0_{m,ADP\leftrightarrow ATP} = 2.5\phi^0_{O_2\leftrightarrow H_2O,NADH} + 1.5\phi^0_{O_2\leftrightarrow H_2O,FADH2}$	
c↔m ATP/ADP transport	$J^{f,0}_{c\ll m,ATP/ADP} = -\left(f^0_{ADP\ll ATP} + f^0_{SCoA\ll Suc}\right)$	*M6*
ATPase (ATP hydrolysis)	$f^0_{c,ATP\ll ADP} = f^0_{13BPG\ll PEP} + f^0_{PEP\ll Pyr} - f^0_{Glc\ll G6P} - f^0_{G6P\ll Gly} - f^0_{F6P\ll F16BP} - 2f^0_{FFA\ll FAC} - J^{f,0}_{c\ll m,ATP/ADP}$	*C20*
c↔m Pi transport	$J^{f,0}_{c\ll m,Pi} = f^0_{ADP\ll ATP} + f^0_{SCoA\ll Suc}$	*M7#*
c↔m CoA transport	$J^{f,0}_{c\ll m,CoA} = f^0_{AKG\ll SCoA} + 7f^0_{FAC\ll ACoA} + f^0_{Pyr\ll ACoA} - f^0_{ACoA\ll Cit} - f^0_{SCoA\ll Suc}$	*M8**
c↔m O_2 transport	$J^{f,0}_{c\ll m,O2} = 0.5*\left(f^0_{O_2\ll H_2O,NADH} + f^0_{O_2\ll H_2O,FADH_2}\right)$	*M1##*
c↔m CO_2 transport	$J^{f,0}_{c\ll m,CO2} = -\left(f^0_{Pyr\ll ACoA} + f^0_{Cit\ll AKG} + f^0_{AKG\ll SCoA}\right)$	*M2***
c↔m H^+ leak	$J^{leak,0}_{c\ll m,H^+} = 10f^0_{O_2\ll H_2O,NADH} + 6f^0_{O_2\ll H_2O,FADH2} - 3f^0_{ADP\ll ATP}$	*M18*
c↔m H^+ transport	$J^{f,0}_{c\leftrightarrow m,H^+} = -\frac{2.303}{\beta_{mit}} C^0_{mit,H^+}(\phi^0_{Pyr\leftrightarrow ACoA} + 7\phi^0_{FAC\leftrightarrow ACoA} + \phi^0_{ACoA\leftrightarrow Cit} + \phi^0_{Mal\leftrightarrow Oxa} - \phi^0_{O2\rightarrow H2O,NADH} - \phi^0_{ADP\leftrightarrow ATP})$	*M17*
b↔c H^+ transport	$J^{f,0}_{b\leftrightarrow c,H^+} = J^{f,0}_{c\leftrightarrow m,H^+} - \frac{2.303}{\beta_{cyt}} C^0_{cyt,H^+}(+\phi^0_{Glc\leftrightarrow G6P} + \phi^0_{F6P\leftrightarrow F16BP} + \phi^0_{GA3P\leftrightarrow 13BPG} - \phi^0_{PEP\leftrightarrow Pyr} - \phi^0_{Pyr\leftrightarrow Lac} + 3\phi^0_{Tgl\leftrightarrow Glr} - \phi^0_{GA3P\leftrightarrow Gr3P} + 2\phi^0_{FFA\leftrightarrow FAC} + \phi^0_{ATP\leftrightarrow ADP} - J^{leak,0}_{c\leftrightarrow m,H^+})$	*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 O_2 transport flux at rest = c↔m O_2 transport flux at rest (Eq. C8).
***b↔c CO_2 transport flux at rest = c↔m CO_2 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

$$C_{ex} + S_{ex} \longleftrightarrow CS_{ex} \quad \text{and} \quad CS_{in} \longleftrightarrow C_{in} + S_{in} \tag{D1}$$

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

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

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

$$[CS]_{ex} + [CS]_{in} + [C]_{in} + [C]_{ex} = [C]_{tot} \tag{D3}$$

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

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

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:

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

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

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

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

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

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

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

where $T_{\max} = D[C]_{tot}/2d_m$ 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 $[S]_{ex} = [S]_{in}$.

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 [ATP]/[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 [NADH]/[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

$$E + \sum_{i=1}^{N_S} \alpha_i S_i \underset{k_{-1}}{\overset{k_{+1}}{\rightleftarrows}} C \underset{k_{-2}}{\overset{k_{+2}}{\rightarrow}} E + \sum_{j=1}^{N_P} \beta_j P_j \qquad \text{(E1)}$$

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: $[E]_{tot}$ = [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

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

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

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

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):

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

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

$$\phi_f = V_{\max}^f \left[\frac{1}{K_m^S} \prod_{i=1}^{N_S} [S_i]^{\alpha_i} \right] \left[1 + \frac{1}{K_m^S} \prod_{i=1}^{N_S} [S_i]^{\alpha_i} \right]^{-1} \tag{E11}$$

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* $\quad \mathrm{Gly}_{n+1} + \mathrm{Pi} \leftrightarrow \mathrm{Gly}_n + \mathrm{G6P}$

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

This is a sum of 2 enzymatic reactions $\mathrm{Gly_{n+1}} + \mathrm{Pi} \leftrightarrow \mathrm{Gly_n} + \mathrm{G1P}$ and $\mathrm{G1P} \leftrightarrow \mathrm{G6P}$ 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_{AMP}/C_{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* $\quad \mathrm{Gly}_n + \mathrm{G6P} + \mathrm{ATP} \leftrightarrow \mathrm{Gly}_{n+1} + \mathrm{ADP} + 2\,\mathrm{Pi}$

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

This is a sum of 4 enzymatic reactions G6P ↔ G1P, G1P + UTP ↔ UDP-Glc + 2 Pi, UDP-Glc + Gly_n ↔ UDP + Gly_{n+1}, and UDP + ATP ↔ UTP + ADP catalyzed by the enzymes *phosphoglucomutase*, *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.

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3. *Hexokinase* $\mathrm{Glc + ATP \leftrightarrow G6P + ADP + H^+}$

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

This is a sum of 2 elementary reactions Glc + Pi ↔ G6P (unfavorable) and ATP ↔ 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).

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4. *Phosphoglucose Isomerase* $\mathrm{G6P \leftrightarrow F6P}$

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

---

5. *Phosphofructokinase* $\mathrm{F6P + ATP \leftrightarrow F16BP + ADP + H^+}$

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

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).

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6. *Glyceraldehyde 3-Phosphate Formation* $\mathrm{F16BP \leftrightarrow 2\ GA3P}$

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

This is a sum of 2 enzymatic reactions F16BP ↔ GA3P + DHAP and DHAP ↔ 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.

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7. *Glyceraldehyde 3-Phosphate Dehydrogenase* $\mathrm{GA3P + Pi + NAD^+ \leftrightarrow 13BPG + NADH + H^+}$

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

8. *Phosphoglycerate Kinase* $\mathrm{13BPG + ADP \leftrightarrow PEP + ATP}$

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

This is a sum of 3 enzymatic reactions 13BPG + ADP ↔ 3PG + ATP, 3PG ↔ 2PG and 2PG ↔ 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* $\mathrm{PEP + ADP + H^+ \leftrightarrow Pyr + ATP}$

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

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* $\mathrm{Pyr + NADH + H^+ \leftrightarrow Lac + NAD^+}$

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

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)* $\mathrm{Pyr \leftrightarrow Ala}$

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

12. *Lipases (Triglycerides Hydrolysis)* $\mathrm{Tgl} \leftrightarrow \mathrm{Glr} + 3\ \mathrm{FFA} + 3\ \mathrm{H^+}$

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

13. *Glycerol 3-Phosphate Dehydrogenase* $\mathrm{GA3P} + \mathrm{NADH} + \mathrm{H^+} \leftrightarrow \mathrm{Gr3P} + \mathrm{NAD^+}$

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

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)* $\mathrm{Gr3P} + 3\ \mathrm{FAC} \leftrightarrow \mathrm{Tgl} + 3\ \mathrm{CoA} + \mathrm{Pi}$

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

This is a sum of 4 enzymatic reactions Gr3P + FAC ↔ LPPA + CoA, LPPA + FAC ↔ PPA + CoA, PPA ↔ DAG + Pi, DAG + FAC ↔ Tgl + 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* $\mathrm{FFA} + \mathrm{CoA} + 2\ \mathrm{ATP} \leftrightarrow \mathrm{FAC} + 2\ \mathrm{ADP} + 2\ \mathrm{Pi} + 2\ \mathrm{H^+}$

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

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)* $\mathrm{ATP} \leftrightarrow \mathrm{ADP} + \mathrm{Pi} + \mathrm{H^+}$

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

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.

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17. *Creatine Kinase*

$$\mathrm{PCr} + \mathrm{ADP} + \mathrm{H}^+ \leftrightarrow \mathrm{Cr} + \mathrm{ATP}$$

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

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.

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18. *Adenylate Kinase*

$$\mathrm{ATP} + \mathrm{AMP} \leftrightarrow 2\ \mathrm{ADP}$$

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

Reactions in Mitochondria

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19. *Pyruvate Dehydrogenase*

$$\mathrm{Pyr} + \mathrm{CoA} + \mathrm{NAD}^+ \leftrightarrow \mathrm{ACoA} + \mathrm{CO_2} + \mathrm{NADH} + \mathrm{H}^+$$

$$\phi_{\mathrm{Pyr}\leftrightarrow\mathrm{ACoA}} = \left[\frac{\dfrac{C_{\mathrm{ADP}}}{C_{\mathrm{ATP}}}}{K^{\mathrm{Ctrl}}_{\mathrm{Pyr}\leftrightarrow\mathrm{ACoA}} + \dfrac{C_{\mathrm{ADP}}}{C_{\mathrm{ATP}}}} \right] \left[\frac{V_{\mathrm{Pyr}\rightarrow\mathrm{ACoA}} \dfrac{C_{\mathrm{Pyr}} C_{\mathrm{CoA}} C_{\mathrm{NAD}^+}}{K_{\mathrm{Pyr}\rightarrow\mathrm{ACoA}}} - V_{\mathrm{Pyr}\leftarrow\mathrm{ACoA}} \dfrac{C_{\mathrm{ACoA}} C_{\mathrm{CO_2}} C_{\mathrm{NADH}} C_{\mathrm{H}^+}}{K_{\mathrm{Pyr}\leftarrow\mathrm{ACoA}}}}{1 + \dfrac{C_{\mathrm{Pyr}} C_{\mathrm{CoA}} C_{\mathrm{NAD}^+}}{K_{\mathrm{Pyr}\rightarrow\mathrm{ACoA}}} + \dfrac{C_{\mathrm{ACoA}} C_{\mathrm{CO_2}} C_{\mathrm{NADH}} C_{\mathrm{H}^+}}{K_{\mathrm{Pyr}\leftarrow\mathrm{ACoA}}}} \right]$$

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_{ADP}/C_{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.

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

$$\mathrm{FAC} + 7\ \mathrm{CoA} + 7\ \mathrm{NAD}^+ + 7\ \mathrm{FAD} \leftrightarrow$$
$$8\ \mathrm{ACoA} + 7\ \mathrm{NADH} + 7\ \mathrm{FADH_2} + 7\ \mathrm{H}^+$$

$$\phi_{\mathrm{FAC}\leftrightarrow\mathrm{ACoA}} = \left[\frac{V_{\mathrm{FAC}\rightarrow\mathrm{ACoA}} \dfrac{C_{\mathrm{FAC}} C_{\mathrm{CoA}} C_{\mathrm{NAD^+}} C_{\mathrm{FAD}}}{K_{\mathrm{FAC}\rightarrow\mathrm{ACoA}}} - V_{\mathrm{FAC}\leftarrow\mathrm{ACoA}} \dfrac{C_{\mathrm{ACoA}} C_{\mathrm{NADH}} C_{\mathrm{FADH_2}} C_{\mathrm{H^+}}}{K_{\mathrm{FAC}\leftarrow\mathrm{ACoA}}}}{1 + \dfrac{C_{\mathrm{FAC}} C_{\mathrm{CoA}} C_{\mathrm{NAD^+}} C_{\mathrm{FAD}}}{K_{\mathrm{FAC}\rightarrow\mathrm{ACoA}}} + \dfrac{C_{\mathrm{ACoA}} C_{\mathrm{NADH}} C_{\mathrm{FADH_2}} C_{\mathrm{H^+}}}{K_{\mathrm{FAC}\leftarrow\mathrm{ACoA}}}} \right]$$

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 acetyletransferase* (*thiolase*) (see Ref. (7), Ch. 21; Ref. (10), Ch. 24).

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21. *Citrate Synthase* $\mathrm{ACoA + Oxa \leftrightarrow Cit + CoA + H^+}$

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

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_{ADP}/C_{ATP}. The enzyme is also known to be inhibited by SCoA which is included as a product inhibitor.

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22. *Aconitase + Isocitrate Dehydrogenase* $\mathrm{Cit + NAD^+ \leftrightarrow AKG + CO_2 + NADH}$

$$\phi_{\mathrm{Cit}\leftrightarrow\mathrm{AKG}} = \left[\frac{\dfrac{C_{\mathrm{ADP}}}{C_{\mathrm{ATP}}}}{K^{\mathrm{Ctrl}}_{\mathrm{Cit}\leftrightarrow\mathrm{AKG}} + \dfrac{C_{\mathrm{ADP}}}{C_{\mathrm{ATP}}}} \right] \left[\frac{V_{\mathrm{Cit}\rightarrow\mathrm{AKG}} \dfrac{C_{\mathrm{Cit}} C_{\mathrm{NAD^+}}}{K_{\mathrm{Cit}\rightarrow\mathrm{AKG}}} - V_{\mathrm{Cit}\leftarrow\mathrm{AKG}} \dfrac{C_{\mathrm{AKG}} C_{\mathrm{CO_2}} C_{\mathrm{NADH}}}{K_{\mathrm{Cit}\leftarrow\mathrm{AKG}}}}{1 + \dfrac{C_{\mathrm{Cit}} C_{\mathrm{NAD^+}}}{K_{\mathrm{Cit}\rightarrow\mathrm{AKG}}} + \dfrac{C_{\mathrm{AKG}} C_{\mathrm{CO_2}} C_{\mathrm{NADH}}}{K_{\mathrm{Cit}\leftarrow\mathrm{AKG}}}} \right]$$

This is a sum of 2 enzymatic reactions Cit ↔ ICit and ICit+NAD^+ ↔ AKG+CO_2+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_{ADP}/C_{ATP}. This reaction is also known to be activated by Ca^{2+} which is not explicitly included in this model of muscle metabolism.

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23. *α-Ketoglutarate Dehydrogenase* $\mathrm{AKG + CoA + NAD^+ \leftrightarrow SCoA + CO_2 + NADH}$

$$\phi_{\mathrm{AKG}\leftrightarrow\mathrm{SCoA}} = \left[\frac{V_{\mathrm{AKG}\rightarrow\mathrm{SCoA}} \dfrac{C_{\mathrm{AKG}} C_{\mathrm{CoA}} C_{\mathrm{NAD^+}}}{K_{\mathrm{AKG}\rightarrow\mathrm{SCoA}}} - V_{\mathrm{AKG}\leftarrow\mathrm{SCoA}} \dfrac{C_{\mathrm{SCoA}} C_{\mathrm{CO_2}} C_{\mathrm{NADH}}}{K_{\mathrm{AKG}\leftarrow\mathrm{SCoA}}}}{1 + \dfrac{C_{\mathrm{AKG}} C_{\mathrm{CoA}} C_{\mathrm{NAD^+}}}{K_{\mathrm{AKG}\rightarrow\mathrm{SCoA}}} + \dfrac{C_{\mathrm{SCoA}} C_{\mathrm{CO_2}} C_{\mathrm{NADH}}}{K_{\mathrm{AKG}\leftarrow\mathrm{SCoA}}}} \right]$$

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24. *Succinyl-CoA Synthetase* $\mathrm{SCoA + GDP + Pi \leftrightarrow Suc + CoA + GTP}$

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

Because the reaction GTP+ADP ↔ GDP+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).

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25. *Succinate Dehydrogenase* $\mathrm{Suc} + \mathrm{FAD} \leftrightarrow \mathrm{Mal} + \mathrm{FADH_2}$

$$\phi_{\mathrm{Suc}\leftrightarrow\mathrm{Mal}} = \left[\frac{V_{\mathrm{Suc}\rightarrow\mathrm{Mal}} \frac{C_{\mathrm{Suc}} C_{\mathrm{FAD}}}{K_{\mathrm{Suc}\rightarrow\mathrm{Mal}}} - V_{\mathrm{Suc}\leftarrow\mathrm{Mal}} \frac{C_{\mathrm{Mal}} C_{\mathrm{FADH_2}}}{K_{\mathrm{Suc}\leftarrow\mathrm{Mal}}}}{1 + \frac{C_{\mathrm{Suc}} C_{\mathrm{FAD}}}{K_{\mathrm{Suc}\rightarrow\mathrm{Mal}}} + \frac{C_{\mathrm{Mal}} C_{\mathrm{FADH_2}}}{K_{\mathrm{Suc}\leftarrow\mathrm{Mal}}}} \right]$$

This is a sum of two enzymatic reactions Suc+FAD↔Fum+$FADH_2$ and Fum↔Mal catalyzed by the enzymes *succinate dehydrogenase and fumarate* (see Ref. (7), Ch. 16; Ref. (10), Ch. 20).

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26. *Malate Dehydrogenase* $\mathrm{Mal} + \mathrm{NAD^+} \leftrightarrow \mathrm{Oxa} + \mathrm{NADH} + \mathrm{H^+}$

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

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27. *Complex I+III+IV* $\mathrm{NADH} + \mathrm{H^+} + 0.5\ \mathrm{O_2} \leftrightarrow \mathrm{NAD^+} + \mathrm{H_2O} + 10\ \Delta\mathrm{H^+}$

$$\phi_{O_2\leftrightarrow H_2O,NADH} = \left[\frac{\exp\left(-\frac{10\Delta G_{\mathrm{H+}}}{RT}\right) V_{O_2\rightarrow H_2O,NADH} \frac{C_{\mathrm{NADH}} C_{\mathrm{O_2}}^{0.5} C_{\mathrm{H^+}}}{K_{O_2\rightarrow H_2O,NADH}} - V_{O_2\leftarrow H_2O,NADH} \frac{C_{\mathrm{NAD^+}}}{K_{O_2\leftarrow H_2O,NADH}}}{1 + \frac{C_{\mathrm{NADH}} C_{\mathrm{O_2}}^{0.5} C_{\mathrm{H^+}}}{K_{O_2\rightarrow H_2O,NADH}} + \frac{C_{\mathrm{NAD^+}}}{K_{O_2\leftarrow H_2O,NADH}}} \right]$$

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 $\Delta G_{\mathrm{H+}} = F\Delta\Psi + RT\ln(C_{\mathrm{H^+_{cyt}}} / C_{\mathrm{H^+_{mit}}})$ where F is the Faraday's constant and $\Delta\Psi$ is the mitochondrial membrane potential.

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28. *Complex II+III+IV* $\mathrm{FADH_2} + 0.5\ \mathrm{O_2} \leftrightarrow \mathrm{FAD} + \mathrm{H_2O} + 6\ \Delta\mathrm{H^+}$

$$\phi_{O_2\leftrightarrow H_2O,FADH2} = \left[\frac{\exp\left(-\frac{6\Delta G_{\mathrm{H+}}}{RT}\right) V_{O_2\rightarrow H_2O,FADH2} \frac{C_{\mathrm{FADH_2}} C_{\mathrm{O_2}}^{0.5}}{K_{O_2\rightarrow H_2O,FADH2}} - V_{O_2\leftarrow H_2O,FADH2} \frac{C_{\mathrm{FAD}}}{K_{O_2\leftarrow H_2O,FADH2}}}{1 + \frac{C_{\mathrm{FADH_2}} C_{\mathrm{O_2}}^{0.5}}{K_{O_2\rightarrow H_2O,FADH2}} + \frac{C_{\mathrm{FAD}}}{K_{O_2\leftarrow H_2O,FADH2}}} \right]$$

This lumped reaction in the electron transport chain involving $FADH_2$-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+}.

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29. *ATP Synthesis (F_1F_0-ATPase or Complex V)* $\mathrm{ADP} + \mathrm{Pi} + \mathrm{H}^+ + 3\Delta\mathrm{H}^+ \leftrightarrow \mathrm{ATP}$

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

ADP is phosphorylated to ATP inside the mitochondrial matrix via the F_1F_0-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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