**Supplementary material and methods**

**Kinetic mass-balance equations**

Intracellular metabolites:

;

Extracellular metabolites:

Rate laws for each flux

1. Glucose transport

1. Glucose utilization

1. Phosphoglucose isomerase activity
2. Phosphofructokinase

1. Aldolase

1. Triosephosphate isomerase

1. Glyceraldehyde phosphate dehydrogenase

,

1. Phosphoglycerate kinase

1. Phosphoglycerate mutase

1. Enolase

1. Pyruvate kinase

1. Lactate dehydrogenase

;

1. Lactate Transport

1. OxPhos

\*

1. Malate-Aspartate shuttle

1. ATPase

1. Creatine kinase

1. Adenylate kinase

1. Oxygen transport to the cell

1. De novo synthesis of Serine through 3-phosphoglycerate dehydrogenase (PHGDH). This involves a lumping of 3 reactions 3PG + NAD+ ↔ p-hPYR + NADH+H+, p-hPYR↔pSER, pSER↔SER

1. De novo GLY synthesis through glycine hydroxymethyltransferase (GHMT, 2.1.2.1). SER↔GLY

**Parameters**

The parameters used in the equations and the justifications for their starting points are shown below.

**Steady state concentrations**

Starting values of model concentrations were taken from a total of several references with values below([Buxton and Frank 1997](#_ENREF_1); [Ercan-Fang, Gannon et al. 2002](#_ENREF_2); [Konig, Bulik et al. 2012](#_ENREF_4)).

|  |  |  |  |
| --- | --- | --- | --- |
| **Steady State Species concentrations** | | | |
| **Species** | **Conc., mM** | **Conc range, mM** | |
| **GLC** | 2.5 | 3.5-6.9 | |
| **G6P** | 0.25 | 0.05-0.32 | |
| **F6P** | 7.73E-02 | 0.01-0.1 | |
| **F16BP** | 1.55E-01 | 0.016-0.030 | |
| **GA3P** | 2.00E-03 | 0.001-0.28 | |
| **DHAP** | 4.14E-02 | 0.01-0.1 | |
| **13BPG\*** | 1.00E-01 |  | |
| **3PG** | 0.5 | 0.05-0.41 | |
| **2PG** | 3.00E-02 | 0.007-0.05 | |
| **PEP** | 0.15 | 0.012-0.27 | |
| **PYR** | 5.00E-01 | 0.02-0.27 | |
| **LAC** | 5 | 0.1-2.5 | |
| **ATP** | 3.00E+00 | 0.5-3.5 | |
| **ADP** | 1.18E-02 | 0.5-1.4 | |
| **AMP** | 4.62E-05 | 0.04 | |
| **Pi** | 4.00E+00 | 3.6-5.7 | |
| **NADH** | 1.00E-03 | 0.03-0.05 | |
| **NAD+** | 5.49E-01 | 0.45 | |
| **PCr** | 1.00E+01 | 10.0-21.0 | |
| **O2i\*\*** | 0.04 | 0.026-0.034 (25mmHg) | |
| **SER** | 3.00E-01 |  | |
| **GLY** | 1.30E-01 |  | |
| **pSER** | 3.00E-01 |  | |
| **pH** | 7.00E+00 |  | |
| **Extracellular concentrations** |
| **GLCe** | 5.00E+00 | |
| **LACe** | 5.00E-01 | |
| **SERe** | 3.00E-01 | |
| **GLYe** | 1.30E-01 | |

\* estimated based on Thermodynamics

\*\* calculated based on Henry's law of solubility of O2 in water at 37**°**C

**Rate constants**

Starting rate constants are shown below. The Km values for substrates in glycolysis are set equal to the steady state substrate concentrations in single-substrate reactions and equal to the product of substrate concentrations in multisubstrate reactions. Km values are reported in units of mM. Vmax values are reported in units of mM/hr.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Enzyme** | **Parameter/value** | |  |  |  |  |  |  |
| **GLUT** | ***Vmaxtr*** | ***Kmglc*** |  |  |  |  |  |  |
|  | 100 | 2.1 |  |  |  |  |  |  |
| **HK** | ***Vmaxf*** | ***Kmf*** | ***Kmr*** | ***Ki*** |  |  |  |  |
|  | 176 | 7.5 | 2.94E-10 | 0.2 |  |  |  |  |
| **PGI** | ***Vmaxf*** | ***Kmf*** | ***Kmr*** |  |  |  |  |  |
|  | 858 | 0.25 | 7.73E-02 |  |  |  |  |  |
| **PFK** | ***Vmaxf*** | ***Kmf*** | ***Kmr*** | ***Ka*** |  |  |  |  |
|  | 1769 | 0.23 | 1.82E-10 | 0.001 |  |  |  |  |
| **ALD** | ***Vmaxf*** | ***Kmf*** | ***Kmr*** |  |  |  |  |  |
|  | 321 | 0.16 | 8.29E-05 |  |  |  |  |  |
| **TPI** | ***Vmaxf*** | ***Kmf*** | ***Kmr*** |  |  |  |  |  |
|  | 859 | 0.04 | 0.002 |  |  |  |  |  |
| **GAPDH** | ***Vmaxf*** | ***Kmnad*** | ***Kmnadh*** | ***Kmgap*** | ***Kmpi*** | ***Kmbpg*** | ***Kmf*** | ***Kmr*** |
|  | 781 | 0.55 | 0.001 | 2.00E-03 | 4 | 1.00E-01 | 4.392e--3 | 1.00E-11 |
| **PGK** | ***Vmaxf*** | ***Kmf*** | ***Kmr*** |  |  |  |  |  |
|  | 221 | 1.18E-03 | 1.5 |  |  |  |  |  |
| **PGM** | ***Vmaxf*** | ***Kmf*** | ***Kmr*** |  |  |  |  |  |
|  | 527.895 | 0.5 | 0.03 |  |  |  |  |  |
| **ENO** | ***Vmaxf*** | ***Kmf*** | ***Kmr*** |  |  |  |  |  |
|  | 1340.089 | 0.03 | 0.15 |  |  |  |  |  |
| **PK** | ***Vmaxf*** | ***Kmf*** | ***Kmr*** | ***Ka*** |  |  |  |  |
|  | 211.525 | 1.77E-03 | 0.15 | 0.5 |  |  |  |  |
| **LDH** | ***Vmaxf*** | ***Kmnadh*** | ***Kmnad*** | ***Kmpyr*** | ***Kmlac*** | ***Kmf*** | ***Kmr*** |  |
|  | 434 | 0.001 | 0.549 | 0.5 | 5 | 5.00E-04 | 2.745 |  |
| **MCT** | ***Vmaxtr*** | ***Kmlac*** |  |  |  |  |  |  |
|  | 60 | 3 |  |  |  |  |  |  |
| **OxPhos** | ***Vmax*** | ***Kmpyr*** | ***Kmo2*** | ***Kmadp*** |  |  |  |  |
|  | 8.4 | 0.001 | 0.005 | 0.005 |  |  |  |  |
| **ATPase** | ***Vmaxf*** | ***Kmf*** | ***Kmr*** |  |  |  |  |  |
|  | 390 | 3 | 4.71E-09 |  |  |  |  |  |
| **CKase** | ***Vmaxf*** | ***Kmf*** | ***Kmr*** |  |  |  |  |  |
|  | 1000 | 5 | 2 |  |  |  |  |  |
| **AKase** | ***Vmaxf*** | ***Kmf*** | ***Kmr*** |  |  |  |  |  |
|  | 2000 | 5 | 2 |  |  |  |  |  |
| **O2transp** | ***ko2*** |  |  |  |  |  |  |  |
|  | 164 |  |  |  |  |  |  |  |
| **PHGDH** | ***Vmaxf*** | ***Km3pg*** | ***Kmnad*** | ***Kmser*** | ***Kmnadh*** | ***Kmf*** | ***Kmr*** |  |
|  | 0 | 0.5 | 0.5 | 0.1 | 0.01 | 0.25 | 1.00E-10 |  |
| **GHMT** | ***Vmaxf*** | ***Kmf*** | ***Kmr*** |  |  |  |  |  |
|  | 0 | 1.5 | 0.5 |  |  |  |  |  |
| ***Other parameters*** | ***nop*** | ***nfad*** | ***nnad*** | ***nox*** |  |  |  |  |
|  | 12.5 | 1.5 | 2.5 | 3 |  |  |  |  |

**Cell uptake and release fluxes**

Typical uptake and release fluxes were considered([Shestov, Mancuso et al. 2013](#_ENREF_6)).

|  |
| --- |
| Cell Uptake-Release Fluxes mM/h |
| Fglc | 80 |
| Fmpc | 8.8 |
| Ftca | 10.7 |
| Fldh | 150 |
| Fltr | 150 |
| CMRO2 | 34 |
| Fatp(cyt) | 150 |
| Fatp(mit) | 174 |
| WE | 14.6 |

**Thermodynamics**

Standard Gibbs Free Energies (dG) were used to constrain fluxes based on Haldane relationships([Goldberg, Tewari et al. 2004](#_ENREF_3); [Li, Wu et al. 2011](#_ENREF_5)).

|  |  |
| --- | --- |
| Reaction | dG, kJ/mol |
| HK | -19.22 |
| PGI | 2.78 |
| PFK | -15.62 |
| ALD | 24.64 |
| TPI | 7.57 |
| GAPDH | 2.6 |
| PGK | -21.6 |
| PGM | 6.35 |
| ENO | -4.47 |
| PK | -27.18 |
| LDH | -23.9 |
| CK | -12.5 |
| AK | 0 |
| ATPase | -32.42 |

**Supplementary References to Materials and Methods**

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