Appendix

Notations:

Two notational conventions are introduced for the following calculations. For a labeling pool A, A denotes the fractional enrichment, i.e.:

$$A = \frac{^{13}A}{[A]}$$

Where ^{13}A is the concentration of ^{13}C labeled molecule A and [A] is the total concentration of molecules A, assumed to be constant in the metabolic steady-state assumption.

The temporal derivation of the concentration of ^{13}C labeled molecule A is written as dA, i.e.:

$$dA = \frac{d^{13}A}{dt}$$

The composite fluxes V_{gt}^{g} and V_{gt}^{n}describing the turnover flux of the first labeled position of glutamate are defined as:

$$V_{gt}^{g} = \frac{V_x^g\, V_{tca}^g}{V_x^g + V_{tca}^g} \quad and \quad V_{gt}^{n} = \frac{V_x^n\, V_{tca}^n}{V_x^n + V_{tca}^n}$$

A) TCA cycle and Glu/Gln cycle modeling

Glial glutamate positions

The position 1 of glial acetyl-CoA is labeled as follows:

$$dAcCoA_1^g = V_{Ace}\ AcO^- - \left(V_{Ace} + V_{dil}^g\right) AcCoA_1^g \quad (1)$$

Where $V_{dil}^{g}$is the dilution flux from the metabolism of unlabeled pyruvate in the glial compartment. Working with the small pool approximation for glial acetyl-CoA (Uffmann and Gruetter 2007) ($dAcCo_1^g \cong 0$ very fast compared with the large glutamate pool), we obtain the following relationship between the fractional enrichment of $AcCo_1^g$ and plasma AcO^- :

$$AcCoA_1^g = \frac{V_{Ace}}{\left(V_{Ace} + V_{dil}^g\right)}\ AcO^- \equiv K_{dil} * AcO^- \quad (2)$$

K_{dil} represents the affinity of glial cells to acetate as metabolic fuel. It was fixed to the value found with ^{13}C nuclear magnetic resonance in a recent study (Duarte *et al*) in similar physiological conditions : $K_{dil} = 0.76$

The position 5 of 2-oxoglutarate labels as follows:

$$dOG_5^g = V_{tca}^g\, K_{dil}\, AcO^- + V_x^g\, Glu_5^g - \left(V_x^g + V_{tca}^g\right) OG_5^g \quad (3)$$

For the position 1 of 2-oxoglutarate, the corresponding equation is:

$$dOG_1^g = \frac{V_{tca}^g}{2}\, OG_5^g + V_x^g\, Glu_1^g - \left(V_x^g + V_{tca}^g\right) OG_1^g \tag{4}$$

The factor $\frac{{V_{tca}}^g}{2}$ is due to the fact that half of the label is flowing from ${OG_5}^g$ to CO_2.The effective flux from ${OG_5}^g$ to ${OG_1}^g$ is $\frac{{V_{tca}}^g}{2}$ (see Figure 2A).

The labeling of glial glutamate at the positions 1 and 5 is given by:

$$dGlu_{1,5}^g = {V_x}^g\, OG_{1,5}^g + V_{nt} Glu_{1,5}^n - ({V_x}^g + V_{nt})\, Glu_{1,5}^g \tag{5}$$

Replacing the isolated ${OG_5}^g$ from (5) in (3):

$$\begin{aligned} dGlu_5^g + \frac{V_x^g}{V_x^g + V_{tca}^g} dOG_5^g \\ = \frac{V_{tca}^g\, V_x^g}{V_x^g + V_{tca}^g} K_{dil}\, AcO^- + \frac{{V_x^g}^2}{V_x^g + V_{tca}^g} Glu_5^g + V_{nt} Glu_5^n - (V_x^g + V_{nt})\, Glu_5^g \end{aligned} \tag{6}$$

Similarly, we replace the isolated OG_1^g from (5) and ${OG_5}^g$ from (5) in (4):

$$\begin{aligned} dOG_1^g = \tfrac{V_{tca}^g}{2} \tfrac{1}{V_x^g} \left(dGlu_5^g - V_{nt} Glu_5^n + (V_x^g + V_{nt}^g)\, Glu_5^g\right) \\ + V_x^g Glu_1^g - \left(V_x^g + V_{tca}^g\right) \tfrac{1}{V_x^g} \left(dGlu_1^g - V_{nt} Glu_1^n + (V_x^g + V_{nt})\, Glu_1^g\right) \end{aligned} \tag{7}$$

$dGlu_5^g$ isolated in equation (6) is replaced in equation (7), which becomes:

$$\begin{aligned} dGlu_1^g + \tfrac{V_x^g}{(V_x^g + V_{tca}^g)} dOG_1^g + \tfrac{V_x^g}{(V_x^g + V_{tca}^g)} \tfrac{V_{tca}^g}{2\, V_x^g} \tfrac{V_x^g}{V_x^g + V_{tca}^g} dOG_5^g \\ = \tfrac{{V_{tca}^g}^2\, V_x^g}{2\,(V_x^g + V_{tca}^g)^2} K_{dil}\, AcO^- + \tfrac{V_{tca}^g {V_x^g}^2}{2\,(V_x^g + V_{tca}^g)^2} Glu_5^g + V_{nt} Glu_1^n - \left(V_{gt}^g + V_{nt}\right) Glu_1^g \end{aligned} \tag{8}$$

The differential of the labeling of the small intermediate pools of the TCA cycles was neglected (Uffmann and Gruetter 2007) compared with the differential of the labeling pools of glutamate ($dOG_1^g, dOG_5^g \ll dGlu_1^g, dGlu_5^g$). Thus, the differential equations of labeling of the positions 5 and 1 of glutamate become:

$$dGlu_5^g = V_{gt}^g\, K_{dil}\, AcO^- + V_{nt} Glu_5^n - (V_{gt}^g + V_{nt})\, Glu_5^g \tag{9}$$

and

$$dGlu_1^g = \tfrac{{V_{tca}^g}^2\, V_x^g}{2\,(V_x^g + V_{tca}^g)^2} K_{dil}\, AcO^- + \tfrac{V_{tca}^g {V_x^g}^2}{2\,(V_x^g + V_{tca}^g)^2} Glu_5^g + V_{nt} Glu_1^n - \left(V_{gt}^g + V_{nt}\right) Glu_1^g \tag{10}$$

Glutamine positions

The labeling of the positions 5 and 1 of glutamine is given by:

$$dGln_{1,5}^{g} = V_{nt}\ Glu_{1,5}^{g} - V_{nt}\ Gln_{1,5} \tag{11}$$

Neuronal glutamate positions

The differential equations of the positions 5 and 1 of the neuronal glutamate are obtained in the same way than for glial glutamate, except that no labeling coming from acetate is entering the neuronal TCA cycle:

$$dGlu_{5}^{n} = V_{nt}Gln_{5} - (V_{gt}^{n} + V_{nt})\ Glu_{5}^{n} \tag{12}$$

$$dGlu_{1}^{n} = \frac{V_{tca}^{n}V_{x}^{n\,2}}{2\,(V_{x}^{n}+V_{tca}^{n})^{2}}Glu_{5}^{n} + V_{nt}Gln_{1} - \left(V_{gt}^{n} + V_{nt}\right)\ Glu_{1}^{n} \tag{13}$$

B) CO_2 labeling

CO_2 is labeled indirectly from AcO^{-}, Glu_{5}^{g}, Glu_{1}^{g}, Glu_{5}^{n} and Glu_{1}^{n} (Figure 2A).

Glial contributions

The input labeling coming from OG_{5}^{g} gives:

$$d^{11}CO_2 = \frac{V_{tca}^{g}}{2}\ OG_{5}^{g} \tag{14}$$

Again, with the assumption of small derivatives of the TCA intermediates compared with the other variables of the differential system, we can extract from equation (3):

$$\left(V_{x}^{g} + V_{tca}^{g}\right) OG_{5}^{g} = V_{tca}^{g}K_{dil}\ AcO^{-} + \ V_{x}^{g}\ Glu_{5}^{g} \tag{15}$$

Substituting then OG_{5}^{g} from equation (15) in equation (14) gives the indirect contribution of labeling of CO_2 from AcO^{-} and Glu_{5}^{g} through OG_{5}^{g}:

$$d^{11}CO_2 = \frac{V_{tca}^{g}}{2}\left[\frac{V_{tca}^{g}}{V_{x}^{g}+V_{tca}^{g}}\ K_{dil}\ AcO^{-} + \ \frac{V_{x}^{g}}{V_{x}^{g}+V_{tca}^{g}}\ Glu_{5}^{g}\right] \tag{16}$$

The input labeling coming from OG_{1}^{g}gives:

$$d^{11}CO_2 = V_{tca}^{g}\ OG_{1}^{g} \tag{17}$$

Working similarly by isolating OG_{1}^{g} in equation (4), we get:

$$d^{11}CO_2 = \frac{V_{tca}^{g}}{V_{x}^{g}+V_{tca}^{g}}\left[K_{dil} * \frac{V_{tca}^{g}}{2}\ \frac{V_{tca}^{g}}{V_{x}^{g}+V_{tca}^{g}}\ AcO^{-} + \frac{V_{tca}^{g}}{2}\ \frac{V_{x}^{g}}{V_{x}^{g}+V_{tca}^{g}}\ Glu_{5}^{g} + V_{x}^{g}Glu_{1}^{g}\right] \tag{18}$$

This is the indirect contribution of labeling of CO_2 from AcO^-, Glu_5^g and Glu_1^g through OG_1^g. Considering both glial labeling through OG_1^g and OG_5^g (16) and (18), we can collect the indirect fluxes coming from AcO^-, Glu_5^g and Glu_1^g.

$$\textbf{\textit{From AcO}}^{-}\textbf{:}\ K_{dil}\ \frac{V_{tca}^g}{2}\ \underbrace{\frac{V_{tca}^g}{V_x^g+V_{tca}^g}}_{\substack{dilution\ though\\ transmitochondrial\\ flux}} + K_{dil}\ \frac{V_{tca}^g}{2}\ \underbrace{\frac{V_{tca}^g}{V_x^g+V_{tca}^g}\frac{V_{tca}^g}{V_x^g+V_{tca}^g}}_{\substack{\textbf{double}\ dilution\ though\\ transmitochondrial\ flux}} \tag{19}$$

$$\textbf{\textit{From Glu}}_{\mathbf{5}}^{\boldsymbol{g}}\textbf{:}\ \frac{V_{tca}^g}{2}\ \underbrace{\frac{V_x^g}{V_x^g+V_{tca}^g}}_{dilution\ in\ OG_5^g} + \frac{V_{tca}^g}{2}\ \underbrace{\frac{V_x^g}{V_x^g+V_{tca}^g}}_{dilution\ in\ OG_5^g}\ \underbrace{\frac{V_{tca}^g}{V_x^g+V_{tca}^g}}_{dilution\ in\ OG_1^g} \tag{20}$$

$$\textbf{\textit{From Glu}}_{\mathbf{1}}^{\boldsymbol{g}}\textbf{:}\ V_{tca}^g\ \underbrace{\frac{V_x^g}{V_x^g+V_{tca}^g}}_{dilution\ in\ OG_1^g} = V_{gt}^g \tag{21}$$

Neuronal contributions

Using the same approach, we get for the indirect fluxes coming from Glu_5^n and Glu_1^n:

$$\textbf{\textit{From Glu}}_{\mathbf{5}}^{\boldsymbol{n}}\textbf{:}\ \frac{V_x^n}{V_x^n+V_{tca}^n} + \frac{V_{tca}^n}{V_x^n+V_{tca}^n}\ \frac{V_x^n}{V_x^n+V_{tca}^n} \tag{22}$$

$$\textbf{\textit{From Glu}}_{\mathbf{1}}^{\boldsymbol{n}}\textbf{:}\ V_x^n\ \frac{V_{tca}^n}{V_x^n+V_{tca}^n} = V_{gt}^n \tag{23}$$

Exchanges with CO_2 dissolved in blood:

Assuming high diffusivity of CO_2 across the blood brain barrier, we obtain a typical CO_2 input flux of 20µmole/g/min (see Methods). In first approximation, we assume that all the CO_2 entering the blood brain barrier is unlabeled. Due to mass conservation at metabolic steady-state, the same CO_2 flux is leaving the brain, in addition to the $3\ V_{tca}^g + 3\ V_{tca}^n$ of CO_2 produced by the brain metabolism. Labeling of CO_2 is given by:

$$d\,{}^{11}CO_2 = V_1\ AcO^- + V_2\ Glu_5^g + V_3\ Glu_1^g + V_4\ Glu_5^n + V_5\ Glu_1^n - \left(3\ V_{tca}^g + 3\ V_{tca}^n + V_{dil}\right)\ {}^{11}CO_2 \tag{24}$$

With V_1, V_2, V_3, $V_4$and V_5 given by the expressions (19), (20), (21),(22) and (23), respectively and $V_{dil} = 20\ \mu mol/g/min$.

References

Duarte JM, Lanz B, Gruetter R Compartmentalized Cerebral Metabolism of [1,6-C]Glucose Determined by in vivoC NMR Spectroscopy at 14.1 T. *Frontiers in neuroenergetics* 3:3.

Uffmann K, Gruetter R (2007) Mathematical modeling of ^{13}C label incorporation of the TCA cycle: the concept of composite precursor function. *Journal of neuroscience research* 85:3304-17.