Elucidating Brain Metabolism by Dynamic ^{13}C Isotopomer Analysis

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Introduction

Metabolic modeling of ^{13}C turnover curves obtained during infusion of a ^{13}C labeled substrate (e.g. [1, 6-$^{13}C_2$]glucose) with a two-compartment neuronal-glial model allows measurement of compartmentalized metabolic fluxes such as the neuronal and glial TCA cycle rates and the rate of glutamate-glutamine cycle. Recently, we reported *in vivo* measurements of time courses for multiple ^{13}C-^{13}C isotopomers, which appear as multiplets in ^{13}C NMR spectra [1]. The goal of the present work was to further extend the isotopomer neuronal-glial metabolic model [2] we recently developed in order to simultaneously and accurately fit multiple ^{13}C isotopomer curves of glutamate, glutamine and aspartate.

Methods

The "standard metabolic model" is the model used in previous studies [2, 3] modified to take into account the additional information from ^{13}C isotopomers. The "extended model" includes additional pools and metabolic fluxes, such as well-established neuronal vesicular and cytosolic glutamate, separate brain pyruvate and lactate pools, glial and neuronal anaplerosis at the level of succinyl-CoA, reversible fluxes between TCA cycle intermediates OAA and succinate (Figure 1). Isotope balance equations were derived for every possible bonded cumomers of glutamate, glutamine and aspartate. This resulted in a set of ~180 differential equations. Solving the set of differential equations yielded time courses for all possible bonded cumomers in glutamate, glutamine and aspartate. Minimization was performed using BFGS or Simplex algorithms. Experimental ^{13}C multiplet time courses of glutamate and glutamine previously measured during [1,6-$^{13}C_2$]glucose infusion in the rat brain [1] was used to fit both models.

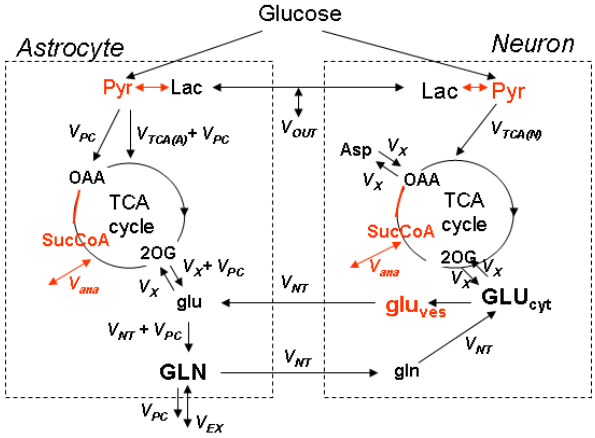

Figure 1: Extended two-compartment metabolic network in the brain where the addition fluxes and pools are shown in red.

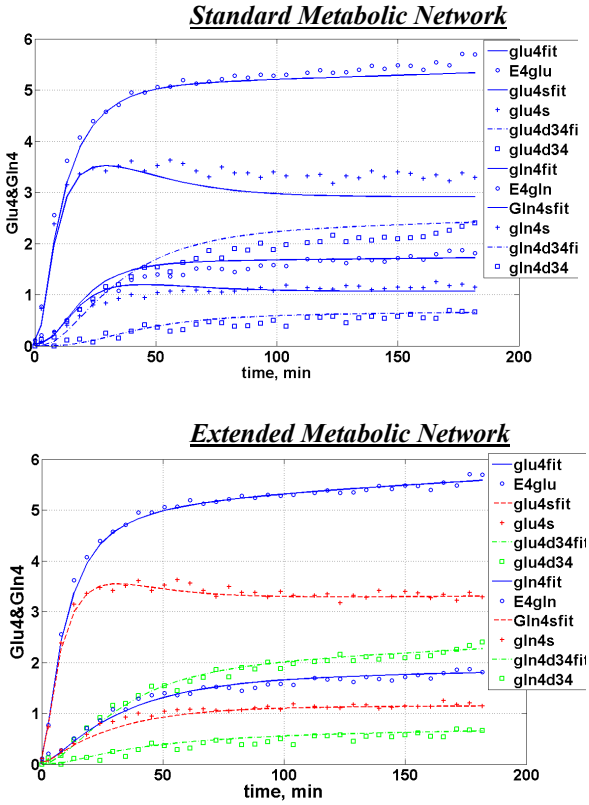

Figure 2: The total glutamate C4 and glutamine C4 labeling curves are the sum of two ^{13}C isotopomer curves corresponding to a singlet C4S and a doublet C4D43. Continuous solid lines represent the best fits to the data using the standard (top) and extended metabolic network (bottom).

Results

When the "standard metabolic model" was used to simultaneously fit the *in vivo* isotopomer turnover curves of glutamate and glutamine, the ^{13}C time courses were not properly fitted as demonstrated for the C4 glutamate and C4 glutamine multiplets (Fig 2 top). Almost all the fitted curves deviated from the experimentally measured time points.

However, with the proposed extended brain metabolic network model, the fit for each isotopomer curve was substantially improved (Fig. 2 bottom). Some of the fitted metabolic fluxes (in µmol/g/min) were: $V_{TCA(n)}$= 0.94 , $V_{TCA(g)}$= 0.41, V_{PC}= 0.08, V_{NT}= 0.08 and V_{ana}= 0.10. In addition, the concentration of the vesicular neuronal glutamate was estimated at 1.5 µmol/g

Discussion

The additional information included in the extended metabolic network allowed simultaneously fitting of at least 11 experimental isotopomers time courses. In addition, the new model allowed the determination of the vesicular glutamate pool. Finally, using the additional information from ^{13}C multiplets leads to an increase in precision for all metabolic fluxes in the model [4].

In summary, we expect that the proposed extended metabolic network model will be useful to further our understanding of brain metabolism and determine metabolic fluxes with improved precision and accuracy.

References

[1] Henry et al. NMR Biomed 16, 400, 2003; [2] Shestov et al ISMRM 2007; [3] Gruetter et al. AJP 2001; [4] Shestov et al ISMRM 2009.

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