A new metabolic model for analysis of dynamic ^{13}C isotopomer time courses in the brain

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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. Current metabolic models typically fit only the total ^{13}C enrichment at each carbon position ("positional model"). Recently, we reported *in vivo* measurements of time courses for multiple ^{13}C-^{13}C isotopomers, which appear as multiplets in ^{13}C spectra [1]. The goals of the present work were (i) to develop a new neuronal-glial metabolic model ("isotopomer model") capable of taking into account the additional information from ^{13}C-^{13}C multiplets and (ii) to determine whether this new model leads to improved precision in fitted metabolic fluxes.

Methods

The metabolic network used was identical to that of previous models [2,3]. Isotope balance equations were derived for every possible isotopomer of glutamate, glutamine and aspartate, including multiply labeled isotopomers. This resulted in a set of ~160 differential equations. Solving the set of differential equations (using the Runge-Kutta algorithm) yielded time courses for all possible isotopomers in glutamate, glutamine and aspartate. Monte-Carlo simulations were performed to evaluate the precision of fitted metabolic parameters with the new model. Fitting of synthetic turnover curves was repeated at least 500 times with a different noise. Minimization was performed using BFGS or Simplex algorithms.

Results

Fig 1 shows examples of experimental time courses for glutamate obtained during C1,6-glucose infusion. Previous metabolic modeling approaches have used only the total ^{13}C positional enrichment (for example GluC4total and GluC3 total) but did not take advantage of the additional information present in ^{13}C-^{13}C multiplets. The new metabolic model presented here allows fitting of each dynamic isotopomer curve (for example GluC4S, Glu-C4D43, Glu-C3S, Glu-C3D, etc). Monte-Carlo simulations show that the new model leads to a significant improvement in the precision of metabolic fluxes (Fig. 2). The SD on V_{NT} decreased from 670% with the positional model to just 19% with the isotopomer model for these particular simulated conditions (t_{max} =150 min, 20 points per turnover curve, noise σ = 0.2 μmol.g^{-1}).

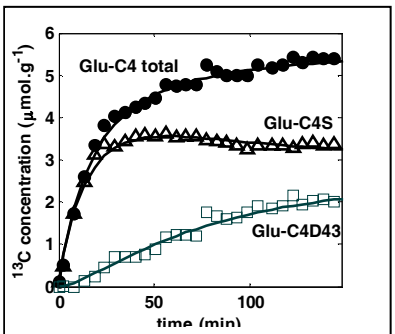

Figure 1. Example of dynamic isotopomer time courses measured in vivo in the rat brain at 9.4 T. The total glutamate C4 labeling curve (Glu-C4 total) is the sum of two ^{13}C isotopomer curves corresponding to a singlet (Glu-C4S) and a doublet (Glu-C4D43). Continuous lines represent the best fits to the data using the new isotopomer metabolic model.

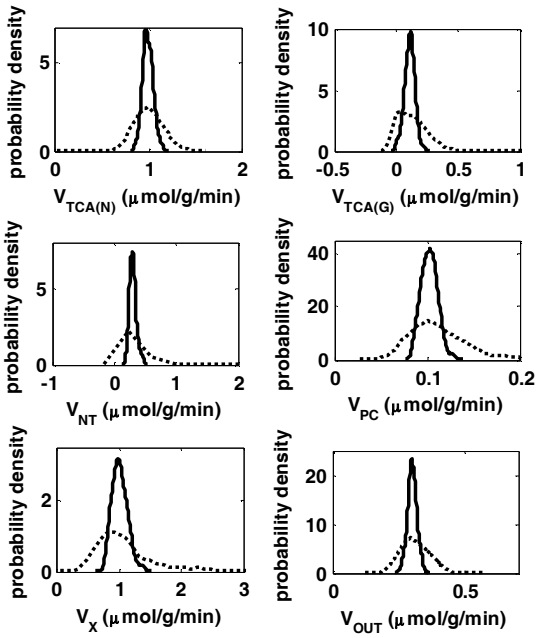

Figure 2. Comparison of the probability distribution of fitted parameters with the positional model (dashed line) and the isotopomer model (continuous line). All six free parameters in the model (V_{PDH}, V_G, V_{NT}, V_{PC}, V_x and V_{OUT}) were determined much more precisely with the isotopomer model.

Discussion

The new metabolic model allowed us to take full advantage of the additional dynamic information available from ^{13}C multiplets in ^{13}C spectra. The isotopomer model can fit up to 20 isotopomer turnover curves compared to 7 positional enrichment curves with the most advanced positional models. Although the computation time was increased, fitting of a single data set still required no more than one minute with a fast personal computer. Using the additional information from ^{13}C multiplets leads to an increase in precision for all six metabolic fluxes in the model. This is consistent with previous studies in the heart which showed that fitting the multiplet turnover curve led to better precision on the determination of V_{TCA} using a one compartment model [4].

Conclusion

In conclusion, we developed a new metabolic model to take into account the additional information from ^{13}C isotopomers, which appear as multiplets in ^{13}C spectra. The additional information leads to significantly increased precision in fitted metabolic fluxes.

References

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