Towards a clinically feasible measurement of TCA cycle rates: a novel approach for mathematical modeling

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Introduction

The incorporation of ^{13}C label from a suitable precursor, such as glucose, into amino acids involved in biochemical reactions for neurotransmission and energy metabolism can be used to study brain metabolism in vivo. The derivation of quantitative metabolic rates traditionally has relied on suitable mathematical models based on a large set of ordinary differential equations (ODE). To quantify metabolic rates the solution of these ODE is varied with the metabolic rates and thus numerically fitted to experimental data of the labeling time courses acquired with ^{13}C MRS [1]. The aim of this study was to derive a mathematical model which allowed to eliminate the need for measuring the enrichment curves of the TCA cycle intermediates for the determination of the TCA cycle flux V_{TCA} and the transmitochondrial flux V_X and to determine if the isotopic enrichment of the C3 and the C4 carbon of glutamate yield sufficient information to determine V_{TCA} without the need to rely on the precursor enrichment time course of pyruvate.

Materials and Methods

Label incorporation into amino acids, namely glutamate (Glu), from a metabolic precursor, e.g.. pyruvate (Pyr), via a single TCA cycle can be described using an established model of label incorporation [1]. With the assumption that the magnitude of the rate of label change of 2-oxoglutarate and oxaloacetate (in µmol/g ^{13}C) is negligible compared to that of glutamate and aspartate, respectively, the terms related to the enrichment of the low concentrated TCA cycle intermediates, which is not measurable, can be eliminated from the set of ODE.

The label incorporation into Glu_4 from Pyr_3 was accurately described by a single ODE $dGlu4/dt = V_{gt}\cdot(Pyr_3/[Pyr]-Glu_4/[Glu])$ (eq. (1)). Likewise the rate of labeling of Glu_3 was given by

$$dGlu_3 = V_{gt}\cdot\frac{x+1}{2x+1}\left(CPF-\frac{Glu_3}{[Glu]}\right) \quad \text{with} \quad CPF = P_X\cdot\frac{Glu_4}{[Glu]}+P_{TCA}\cdot\frac{Pyr_3}{[Pyr]} = \frac{V_X}{V_x+V_{TCA}}\cdot\frac{Glu_4}{[Glu]}+\frac{V_{TCA}}{V_x+V_{TCA}}\cdot\frac{Pyr_3}{[Pyr]} \quad \text{, eq. (2)}$$

where $x = V_x/V_{TCA}$. The composite precursor function (CPF) is the driving input function of the ODE. For a more intuitive insight the expressions for CPF can be interpreted as probabilities, where coefficients P_X and P_{TCA} represent the probability that label is received by Glu_4 or Pyr_3.

Furthermore the resulting ODE can be solved analytically [2]. The labeling curve of Glu_4 [eq. (1)] can sufficiently well approximated by function $Glu_4/[Glu] = y_0 + IE_0\cdot(1-\exp(-t/\tau))$, where the time constant $k = [Glu]/V_{gt}$ is the turnover time. Involved fluxes are represented by the composite rate $V_{gt} = V_X\cdot V_{TCA}/(V_X+V_{TCA})$. The ODE of the enrichment of Glu_4 was used to substitute the expressions for labeling of Pyr_3 in the ODE for labeling of Glu_3 [eq. (2)]. A numerical fit provided a value of τ, which allows for determination of V_X and V_{TCA} using the ODE describing the isotopic enrichment of the C3 of glutamate (Glu_3). Enrichment curves were acquired from Sprague-Dawley rats using a protocol similar to elsewhere [3]. The experiment was conducted on an actively-shielded 9.4T spectrometer (Magnex, Oxford, UK) using a DEPT sequence with an ISIS localization scheme and outer volume suppression.

Results and Discussion

Fig. 1 shows the measured time enrichment course of Glu_4 and Glu_3 (squares and diamantes, respectively) as derived from the acquired 13C spectra (Fig. 2). The results from the fit of the turnover curves of Glu_4 and Glu_3 are shown as straight and dashed line, respectively. The resulting time constant τ and the parameter y_0 were used as a constrain for the fit of the Glu_3 enrichment curve with an analytical solution of eq. (1), which has the form $y = A + B\cdot\exp(-\tau\cdot t) + C\cdot\exp(\lambda\cdot t)$. From $\lambda = (x+1)/(2x+1)\ V_{TCA}/[Glu]\ V_{TCA}$ ranged from 0.48 to 0.71 µmol/(g·min) for $x = 1$ and $x >> 1$. This range of V_{TCA} corresponds to values found in the literature [2,3] derived here without measuring the precursor turnover.

Fig. 1

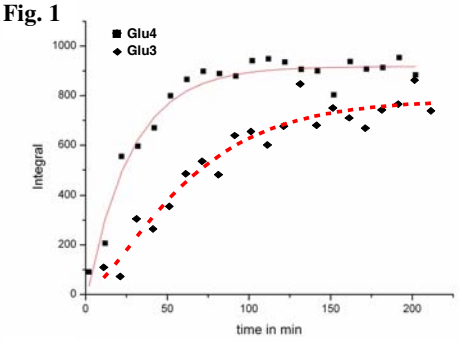
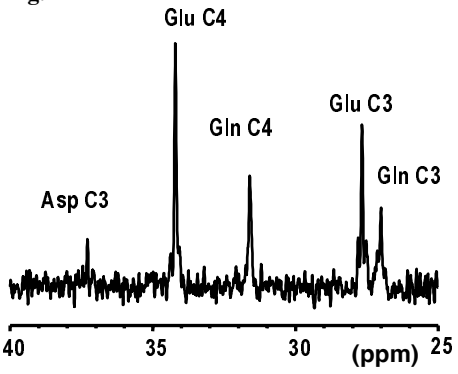


Fig. 2



Conclusions

The presented model allowed for derivation of two equations describing the isotopic enrichment into Glu_3 and Glu_4, which was analytically solved. With a fit of the analytical solutions to experimental data the turnover time constant of the labeling was determined to further determine both V_X and V_{TCA}. For a precise determination of V_X, we found that a high SNR at the initial time points is required. This approach not only eliminates terms of the TCA cycle intermediates from the model, but also provides a framework to study turnover rates without any knowledge about the labeling of the precursor pyruvate.

We conclude that a mathematical approach is feasible that allows for an explicit solution of the labeling curve for Glu3, based on the measured time course of Glu4 which permits (a) the elimination of the need to know the precursor enrichment and (b) allows for explicit curve fitting.

References

[1] Gruetter et al., AJP 2001; [2] Choi et al., AJP 2003 ; [3] Henry et al., MRM 2003

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