Combination of Datasets from [2-^{13}C]Acetate and [1-^{13}C]Glucose Experiments Improve Accuracy of Metabolic Rates Determination in Humans

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Introduction Since 1992, NMR spectroscopy (MRS) with infusions of ^{13}C-labeled substrates have allowed studies of metabolic pathways, including the astroglial and neuronal TCA cycle (V_{TCAa}, V_{TCAn}) and glutamatergic neurotransmission (V_{NT}) in human brain *in vivo* [1]. Most studies have used a two-compartment metabolic model and numerical algorithms to estimate the rates from ^{13}C-labeling time courses of amino acids such as glutamate and glutamine during ^{13}C-labeled glucose infusions [2-7]. However, there are limitations to the glucose approach due to incorporation of label both in the astroglia and neurons (3). Furthermore, based on numerical simulations, Shestov et al. [8] have argued that below a S/N threshold, the glucose measurement of V_{NT} may be unreliable (7). An alternate labeled substrate for studying brain metabolism is [2-^{13}C]acetate, which has the advantage of incorporation only into glia [3]. However dynamic acetate studies in humans have not been performed. In this abstract we report results at 4T from human occipital lobe, combining ^{13}C MRS data from paired [1-^{13}C]glucose and [2-^{13}C]acetate infusions to improve the quantitative determination of these metabolic rates. We find that the double infusion method provides a significant improvement in the reliability of determinations brain metabolic fluxes over either isotopic precursor on its own.

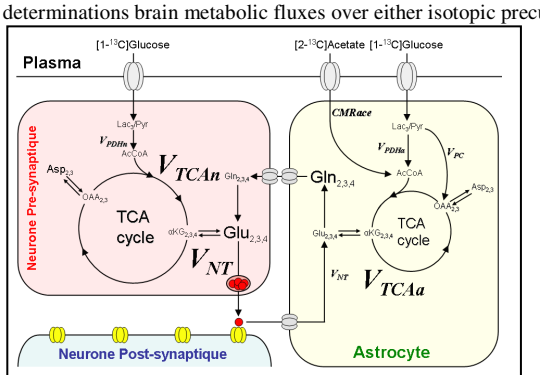

Fig. 1. Two-compartment metabolic model. Left: neuronal compartment; Right: astroglial compartment.

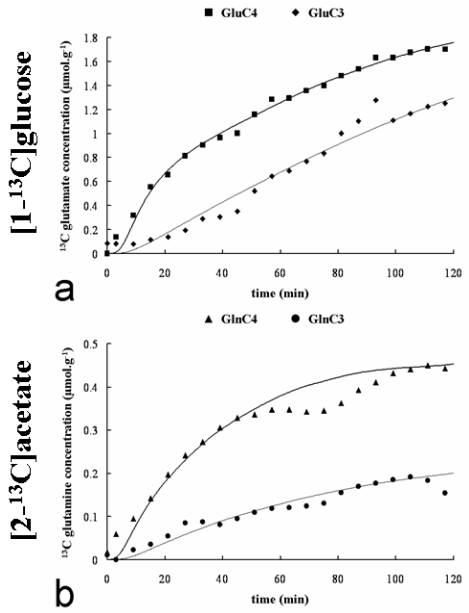

Fig. 2. Typical ^{13}C glutamate (A) and glutamine (B) time-courses and best fits (C4 and C3 positions) obtained during (A) [1-^{13}C]glucose or (B) [2-^{13}C]acetate infusions.

Materials and Methods

MRS acquisition. [1-^{13}C]glucose and [2-^{13}C]acetate infusion experiments were conducted in 8 healthy volunteers (3 females and 5 males; aged 26±7 years, BMI 23±4 kg/m^2; mean ± SD), yielding 16 pairs of ^{13}C NMR studies. Subjects lay supine in a 4.0 T whole-body magnet (Bruker Instruments, Billerica, MA) with the head lying on top of one 8.5-cm-diameter ^{13}C circular coil and two ^{1}H quadrature coils for ^{1}H acquisition and decoupling. After tuning, acquisition of scout images, shimming with the FASTERMAP procedure [9], and calibration of the decoupling power, ^{13}C MRS spectra were acquired with polarization transfer [2] from a 50x40x45 mm^3 volume located in the occipital-parietal lobe before and during infusions of [1-^{13}C]glucose or [2-^{13}C]acetate (TR=2500ms, 128 transients). Infusion protocols for the [1-^{13}C]glucose and [2-^{13}C]acetate are described in detail elsewhere [2,3]. During the acquisition, blood samples were collected every 5-10 minutes to measure acetate, glucose and lactate plasmatic concentration and ^{13}C fractional enrichment.

MRS spectral analysis. Before processing, ^{13}C scans were added in running averages of 15 min. All spectra were analyzed using LCModel 6.1 [10] with simulated basis sets and modified input parameters as described by Henry et al. [11]. Concentrations for different isotopomers of glutamate and glutamine were obtained relative to the natural abundance signal of NAA assuming a concentration of 11µmol.g^{-1}. The concentrations of glutamate and glutamine used for the modeling were assumed to be 9.1 and 4.1 µmol.g^{-1}, respectively, similar to values reported previously by MRS in a similar volume [12].

Metabolic modeling analysis. Paired sets of ^{13}C time courses of Glu and Gln C4, C3, and C2 from [1-^{13}C]glucose and [2-^{13}C]acetate infusion experiments were fitted together or separately with a neuronal-astrocytic model (Fig. 1) [2-7]. Metabolic modeling was done with Matlab 7.0 (The MathWorks Inc., Natick, MA) and CWave [13] using time-courses for plasma acetate, lactate and glucose concentrations and ^{13}C fractional enrichments as inputs functions for each experiment. Values for V_{NT}, V_{TCAn}, and V_{TCAa} were adjusted iteratively with simulated annealing. Because no consensus has been found on the value of the α-ketoglutarate/glutamate exchange rate (V_X), it was fitted as well.

Evaluation of fit reliability. Probability distributions for V_{TCAn}, V_{TCAa} and V_{NT} were estimated with Monte-Carlo simulations when considering the ^{13}C-labeling time-courses from (i) only [1-^{13}C]glucose, (ii) only [2-^{13}C]acetate and (iii) both (Fig. 2). Distributions were calculated for each data set.

Results and Discussion

The volumes of interest contained 47±1% grey matter. The metabolic rates were consistent with values reported previously for human brain from either [1-^{13}C]glucose or [2-^{13}C]acetate infusions [1-7]: V_{NT}=0.16±0.02, V_{TCAn}=0.53±0.03, and V_{TCAa}=0.13±0.01 µmol.g^{-1}.min^{-1} (mean±SD, n=8). Table 1 shows that the standard deviations for all metabolic rates were smaller when glucose and acetate data were combined. However, neuronal and astroglial V_{TCAn} and V_{TCAa} were still well-determined when the ^{13}C-labeling time courses from [1-^{13}C]glucose and [2-^{13}C]acetate experiments are considered separately. Regarding V_{NT}, data from [2-^{13}C]acetate infusions are comparatively more reliable [8]. However, the precision calculated here for [1-^{13}C]glucose (36%) much tighter than what has been reported [8].

	[1-^{13}C]Glc	[1-^{13}C]Ace	Both
V_{NT}	36 ± 5	16 ± 2	5 ± 1
V_{TCAa}	27 ± 8	9 ± 7	12 ± 4
V_{TCAn}	9 ± 5	19 ± 3	7 ± 2

Table 1. Percentage standard deviations for V_{NT}, V_{TCAn} and V_{TCAa} for ^{13}C time courses from only [1-^{13}C]glucose, only [2-^{13}C]acetate, and both (mean SD ± standard deviation of the SD (n=8). The percent SD was determined for each data set using Monte Carlo iterations.

Conclusion

In this study, ^{13}C MRS data from paired [1-^{13}C]glucose and [2-^{13}C]acetate infusions were combined to improve the quantitative determination of V_{NT}, V_{TCAn}, and V_{TCAa}. The results demonstrate that our approach provides more accurate quantification of metabolic rates in human brain. However, under our experimental conditions good precision and accuracy for all rates was obtained with either tracer on its own, validating the conclusions from previous studies. However the enhanced precision of the combined method should greatly facilitate the application of ^{13}C MRS to study changes in brain metabolism and neurotransmission in a variety of experimental conditions such as disease and drug administration.

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