Quantification of High-Resolution ^{1}H-[^{13}C] NMR Spectra from Rat Brain Extracts

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ABSTRACT

NMR spectroscopy in combination with ^{13}C-labeled substrate infusion is a unique technique to obtain information about dynamic metabolic fluxes non-invasively *in vivo*. In many cases the *in vivo* information content obtained during dynamic ^{13}C studies in rodents can be enhanced by high-resolution ^{1}H-[^{13}C] NMR spectroscopy on brain extracts. Previously it has been shown that ^{1}H NMR spectra from rat brain extracts can be accurately quantified with a spectral fitting routine utilizing simulated basis sets using complete prior knowledge of chemical shifts and scalar couplings. The introduction of ^{13}C label into the various metabolites presents complications that demand modifications of the spectral fitting routine. As different multiplets within a given molecule accumulate various amounts of ^{13}C label, the fixed amplitude relationship between multiplets typical for ^{1}H NMR spectra must be abandoned. In addition, ^{13}C isotope effects lead to spectral multiplet patterns that become dependent on the amount of ^{13}C label accumulation, thereby preventing the use of a common basis set. Here a modified spectral fitting routine is presented that accommodates variable ^{13}C label accumulation and ^{13}C isotope effects. Spectral fitting results are quantitatively compared to manual integration on column-separated samples in which spectral overlap is minimized.

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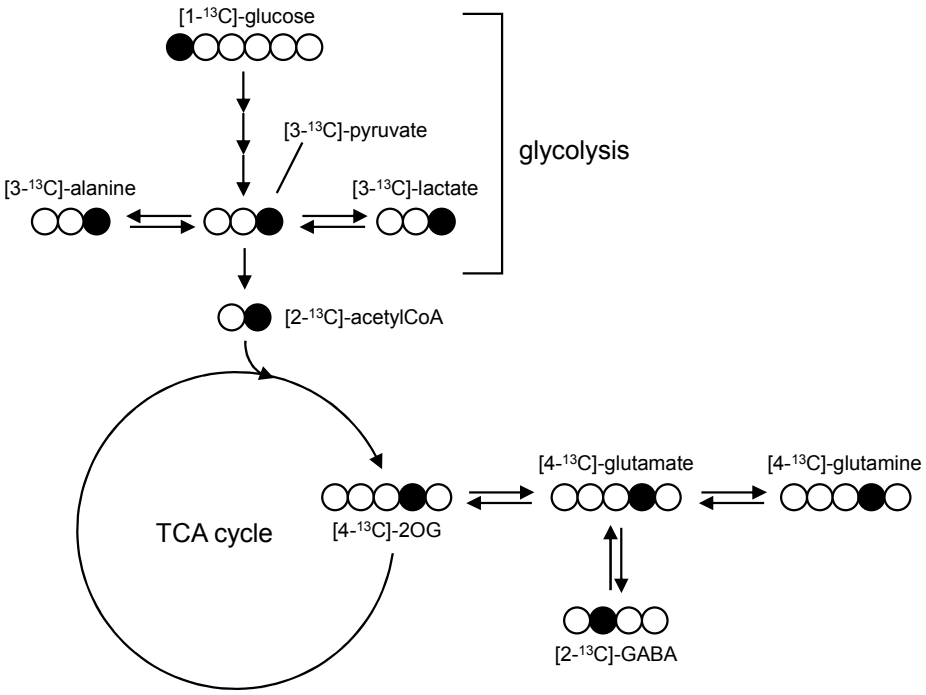

Figure S1. Metabolic model of glycolysis and the tricarboxylic acid (TCA) cycle. Small circles correspond to carbon atoms, whereby filled circles indicate ^{13}C labeled positions. When [1-^{13}C]-glucose is used as the substrate, the ^{13}C label is transferred via the glycolytic pathway to [3-^{13}C]-pyruvate. Pyruvate is in fast exchange with alanine and lactate, both of which are detectable by ^{1}H and ^{1}H-[^{13}C] NMR spectroscopy. The ^{13}C label enters the TCA cycle when [2-^{13}C] acetyl CoA condenses with oxaloacetate to form [2-^{13}C]-citrate. After three additional TCA cycle steps the ^{13}C label arrives in 2-oxoglutarate (2OG) which is in fast exchange with the large, NMR detectable pool of glutamate. During the first turn of the TCA cycle [4-^{13}C]-glutamate is formed. Subsequent turns lead to the formation of [2-^{13}C] and [3-^{13}C]-glutamate. In the brain the excitatory neurotransmitter glutamate can be converted to glutamine via the glutamate-glutamine neurotransmitter cycle or to the inhibitory neurotransmitter γ-amino butyric acid (GABA). The metabolic model shown is in essence a one-compartment model. In many applications related to cerebral metabolism the model is extended to four compartments encompassing blood, astroglia, glutamatergic neurons and GABAergic neurons.

Table S1: Carbon-13 fractional enrichments as determined by spectral fitting of non-separated or by integration of column-separated brain extract samples[1].

infusion time (min)	15	15	30	60	60
multiplet	integration/fitting	integration/fitting	integration/fitting	integration/fitting	integration/fitting
Asp-H3	15.2 / 14.6	16.1 / 19.2	20.2 / 22.4	27.4 / 26.1	36.6 / 36.9
Ala-H3	26.9 / 28.2	30.8 / 30.4	30.9 / 32.2	37.9 / 36.6	32.1 / 33.6
Lac-H3	27.2 / 27.0	32.1 / 33.9	30.8 / 28.3	36.7 / 37.6	34.4 / 34.8
Gln-H3	5.4 / 5.4	5.1 / 5.4	8.7 / 10.5	19.5 / 20.1	17.5 / 18.2
Gln-H4	12.0 / 11.5	11.9 / 12.3	20.3 / 19.1	32.2 / 33.2	25.7 / 24.2
Glu-H3	8.6 / 8.9	8.7 / 8.9	16.8 / 16.5	27.2 / 27.0	29.6 / 27.0
Glu-H4	26.9 / 26.0	25.8 / 26.2	34.0 / 32.6	38.6 / 37.9	37.1 / 37.2
GABA-H2	20.4 / 19.1	18.3 / 18.7	27.2 / 24.0	37.2 / 36.6	35.5 / 32.8
GABA-H3	7.8 / 6.7	7.5 / 7.2	12.6 / 12.7	28.9 / 29.3	25.1 / 23.6

[1] Metabolite fractional enrichments are not corrected for ^{13}C blood glucose fractional enrichment.