Study Design

^{13}C isotopomer analysis can be utilized to perform flux analysis in both isotopic steady and non-steady states. Isotopic steady state is reached when there is no measurable change in ^{13}C incorporation in metabolites even in the continual presence of ^{13}C labeled substrates. NMR-based estimation of metabolic fluxes require that labeled substrates are able to probe the primary pathways of interest without interfering with each other. One strategy to discriminate between glucose and fatty acids as sources of acetyl-CoA in cell culture is detailed below.

Glucose vs. Fatty acid flux

1. An approach to probe glucose and fatty acid flux is to use ^{13}C labeled glucose and a surrogate for fatty acids, acetate.
2. Glucose oxidation generates pyruvate, which is converted by pyruvate dehydrogenase to acetyl-CoA. In this conversion, C-1 of pyruvate is lost as carbon dioxide and ^{13}C labels from C-2 and C-3 are propagated.
3. Acetyl-CoA synthetase catalyzes the conversion of acetate to acetyl-CoA. This conversion preserves the acetate labeling pattern. Note that acetate is freely permeable to the cell membrane. If regulation at the level of carnitine palmitoyl transferase (CPT) is a target of the study, the appropriate long chain fatty acid should be used instead.
4. ^{13}C label from acetyl-CoA is transferred to glutamate C-4 and C-5. Other carbons of glutamate gain ^{13}C incorporation in the subsequent turns of TCA cycle due to the catalytic regeneration of oxaloacetate that is a substrate for condensation with acetyl-CoA on the next turn of the cycle.
5. To distinguish between glucose and fatty acid utilization, a combination of [1, 6 – $^{13}C_2$]glucose (producing C2 labeled acetyl-CoA) and [1, 2 – $^{13}C_2$] acetate (producing [1,2-$^{13}C_2$]acetyl-CoA) can be utilized. The selectivity
6. [1, 6 – $^{13}C_2$] glucose generates [2 – ^{13}C] pyruvate and subsequently, [4 – ^{13}C] glutamate on first turn of CAC (**Fig 1a**). Similarly, [1, 2 – $^{13}C_2$] acetate generates [4, 5 – $^{13}C_2$] glutamate (**Fig 1b**).

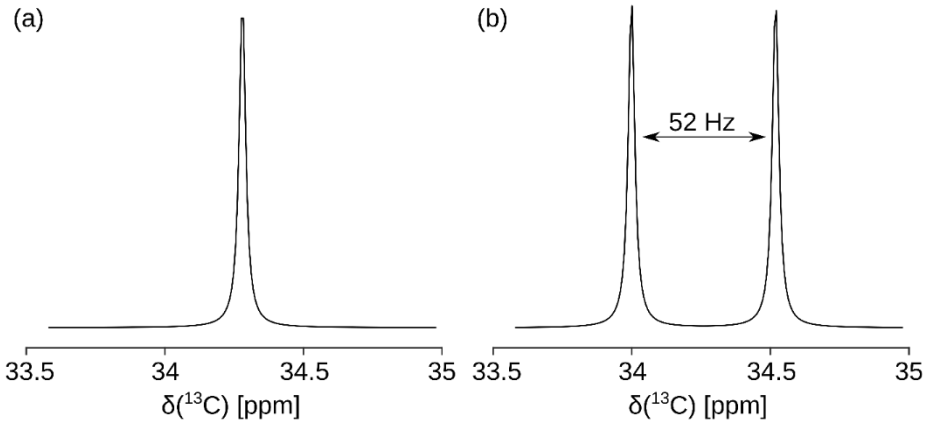

Figure 1*: Simulated ^{13}C spectra of C-4 glutamate after 1st turn of citric acid cycle arising from (**a**) [1, 6 – $^{13}C_2$]glucose and (**b**) [U-^{13}C]fatty acids. [1, 6 – ^{13}C]glucose generates methyl labeled acetyl-CoA which labels C-4 of glutamate resulting in a singlet. Similarly, [U – ^{13}C]fatty acids generate doubly labeled acetyl-CoA which labels positions 4 & 5 of glutamate. This results in a doublet at C-4 position with J_{CC} of 52 Hz*

7. In the subsequent turns of citric acid cycle, glucose oxidation gives rise to a strong doublet (C4D34; Fig 2a) with 34 Hz split in the glutamate C-4 cluster. Fatty acid oxidation gives rise to a strong quartet (C4Q; Fig 2b).

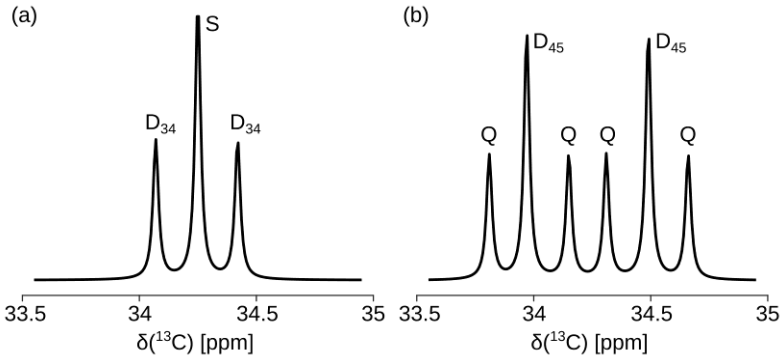

Figure 2: *Simulated ^{13}C Spectra of C-4 glutamate at isotopic steady state arising from (**a**) [1, 6 – $^{13}C_2$]Glucose and (**b**) [U-^{13}C]fatty acids. Simulations assume pyruvate dehydrogenase flux supplies 50% of acetyl-CoA entering citric acid cycle flux. Isotopomers D_{34}, D_{45} and Q represent the pools of glutamate labeled in carbons 3 & 4, carbons 4 & 5 and carbons 3, 4 & 5.*

8. These peak areas can be used to obtain non-steady state estimates of fractional enrichment of acetyl-CoA from different sources ***(1)***. Further details of analysis are discussed in the metabolic modelling section.

Refs

1. Malloy CR, Thompson JR, Jeffrey FMH, et al (1990) Contribution of exogenous substrates to acetyl coenzyme A: measurement by carbon-13 NMR under non-steady-state conditions. Biochemistry (Mosc) 29:6756–6761