Supporting Information

Characterization of kinetic isotope effects and label loss in deuterium-based isotopic labeling studies.

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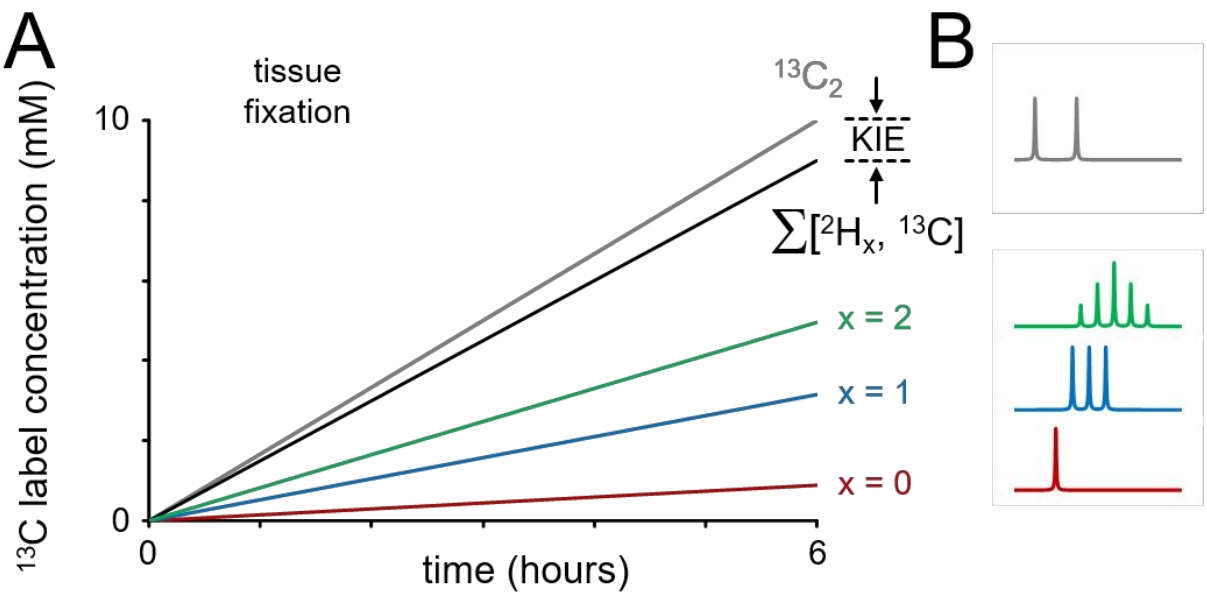

Figure S1 – Illustration of the strategy used to detect deuterium label loss and kinetic isotope effects during glucose metabolism in RG2 cancer cells. The metabolic fate of [6,6-$^2H_2$, 6-^{13}C]-glucose in RG2 cells is similar to that in normal brain, up to and including lactate formation (Fig. 1A). However, unlike normal brain the intracellular lactate is expelled to the extracellular space when it accumulates linearly over time (A). The ^{2}H label loss can be quantitatively determined from the unique spectral patterns of non (x=0), single (x=1) and double (x=2)-deuterated compounds, similar to that in rat brain (Fig. 1B/C). However, while the ability to measure kinetic isotope effects (KIEs) in rat brain is limited to early time points (Fig. 1B), the ability to measure KIEs in lactate produced by RG2 cancer cells improves over time as the extracellular lactate concentration and thus the NMR sensitivity increases over time. In the current study, KIEs on lactate were determined six hours after the administration of [6,6-$^2H_2$, 6-^{13}C] and [5,6-$^{13}C_2$]-labeled glucose.

Table S1

Table S1. ^{13}C chemical shifts and ^{2}H-^{13}C and ^{13}C-^{13}C scalar coupling constants

chemical moiety	chemical shift (ppm)	spectral pattern [1]	^{2}H-^{13}C coupling (Hz)	^{13}C-^{13}C coupling (Hz)
[2-^{13}C]-acetate	24.10	singlet	–	–
[2-$^{3}H_2$, 2-^{13}C]-acetate	23.39	septet	19.4	–
[1,2-$^{13}C_2$]-acetate	24.10	doublet	–	52.2
[6-^{13}C]-α-glucose	61.26	singlet	–	–
[6-^{13}C]-β-glucose	61.40	singlet	–	–
[6,6-$^{2}H_2$, 6-^{13}C]-α-glucose	60.63	quintet	21.6	–
[6,6-$^{2}H_2$, 6-^{13}C]-β-glucose	60.77	quintet	21.6	–
[5,6-$^{13}C_2$]-α-glucose	61.26	doublet	–	43.3
[5,6-$^{13}C_2$]-β-glucose	61.40	doublet	–	43.1
[4-^{13}C]-glutamate	34.20	singlet	–	–
[4-^{2}H, 4-^{13}C]-glutamate	33.90	triplet	19.3	–
[4,4-$^{2}H_2$, 4-^{13}C]-glutamate	33.60	quintet	19.3	–
[4,5-$^{13}C_2$]-glutamate	34.20	doublet	–	51.3
[4-^{13}C]-glutamine	31.70	singlet	–	–
[4-^{2}H, 4-^{13}C]-glutamine	31.42	triplet	19.1	–
[4,4-$^{2}H_2$, 4-^{13}C]-glutamine	31.15	quintet	19.1	–
[4,5-$^{13}C_2$]-glutamine	31.70	doublet	–	48.4
[3-^{13}C]-lactate	21.00	singlet	–	–
[3-^{2}H, 3-^{13}C]-lactate	20.73	triplet	19.6	–
[3,3-$^{2}H_2$, 3-^{13}C]-lactate	20.46	quintet	19.6	–
[2,3-$^{13}C_2$]-lactate	21.00	doublet	–	36.7

[1] 1 : 1 : 1 triplet intensity ratio, 1 : 2 : 3 : 2 : 1 quintet intensity ratio, 1 : 3 : 6 : 7 : 6 : 3 : 1 septet intensity ratio

Table S2

Table S2: overview of ^2H detection following [6,6-$^2H_2$]-glucose and [$^2H_3$]-acetate administration

[1] ^2H detection as percent of theoretical condition without ^2H label loss; [2] correction factor to account for ^2H label loss (= 100/"^2H detection %").

[6,6-$^2H_2$]-glucose		Number of ^2H's	Lactate (%)	Glutamate (%)	Glutamine (%)
		0	6.1	8.6	17.1
		1	19.2	58.5	48.9
		2	74.7	32.9	34.0
	^2H detection[1] (%)		84.3	62.2	58.5
	Corr. Factor[2]		1.19	1.61	1.71
[$^2H_3$]-acetate		0	-	1.9	1.7
		1	-	25.0	23.9
		2	-	73.1	74.4
	^2H detection[1] (%)			85.6	86.4
	Corr. Factor[2]			1.17	1.16

Table S3

[6,6-2H2]-glucose				[2H3]-acetate			
GluC45	GluC4*	GlnC45	GlnC4*	GluC45	GluC4*	GlnC45	GlnC4*
258.1359	243.137	70.87629	90.79144	52.47899	50.7415	132.9089	122.9795
258.5385	266.4078	68.76839	80.29604	51.1376	57.2921	122.9637	104.16
271.24	253.5845	69.21154	62.13724	51.81434	57.4459	131.2125	131.575
260.6389	244.5796	98.10261	83.25074	53.23753	51.7202	127.1388	143.4107
264.6017	223.7511	74.96709	59.51254	53.66343	48.1937	132.2286	128.4037
263.5571	264.5288	107.0283	96.12124	52.51301	50.2862	127.3109	114.6129
256.8924	248.7913	82.67671	86.61864	53.3834	52.1368	126.9423	117.5223
256.6564	245.2777	97.54357	83.02174	51.30678	46.98212	121.6785	104.2648
235.7482	254.5008	78.16225	70.81884	49.36228	41.59322	135.0201	114.5811
256.1952	243.6042	98.21196	115.3378	52.75761	55.9971	133.5952	121.2488
256.8988	245.2395	94.74825	76.70464	55.09579	52.2748	107.6864	120.8888
256.7068	246.6887	79.35503	90.37984	53.69655	57.1855	132.1521	127.8991
256.9639	245.0671	64.49319	86.74694	53.87924	45.7634	125.4612	120.9993
259.4076	248.8593	96.40961	104.994	52.55123	50.3653	134.3086	122.6068
260.0252	238.5266	88.45408	72.32584	56.21894	50.8962	128.6388	111.9608
237.6358	263.5782	61.37202	75.96994	53.61451	40.4319	129.203	122.5179
257.1994	248.4743	80.304	65.61784	53.22721	49.516	137.6641	133.4531
259.0861	223.3834	93.77139	58.78034	50.7847	51.02	131.7984	127.8295
255.5556	245.7601	91.28173	89.13214	48.53252	39.7964	128.7336	126.9568
257.7725	263.5441	96.20887	84.04474	48.5022	50.20141	127.2967	102.3166

Quantification of glutamate (Glu) and glutamine (Gln) peaks from the spectra generated via Monte Carlo simulation, for [6,6-2H2]-glucose, and [2H3]-acetate.