Supplemental Information

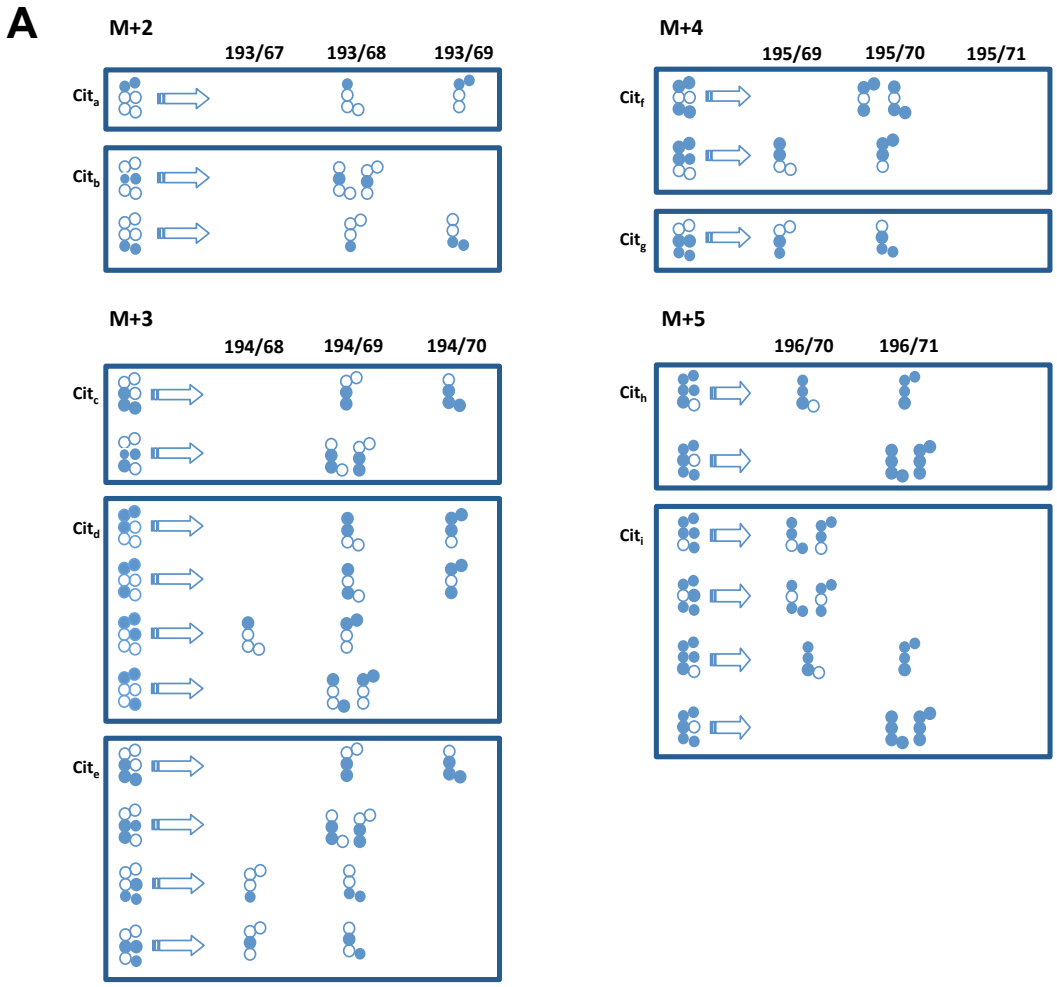
Supplemental Figures

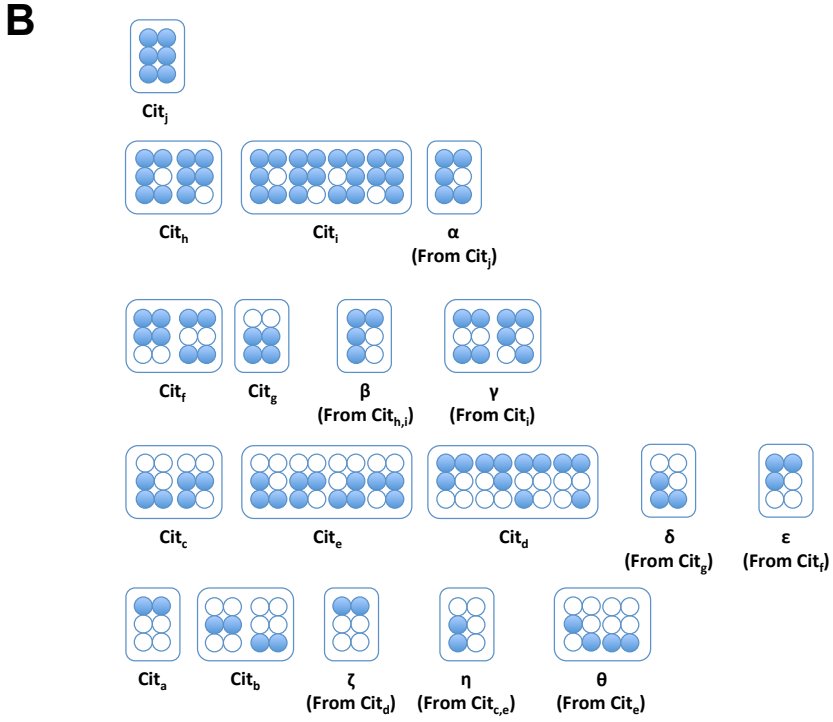
Supplemental Tables

Supplemental Experimental Methods

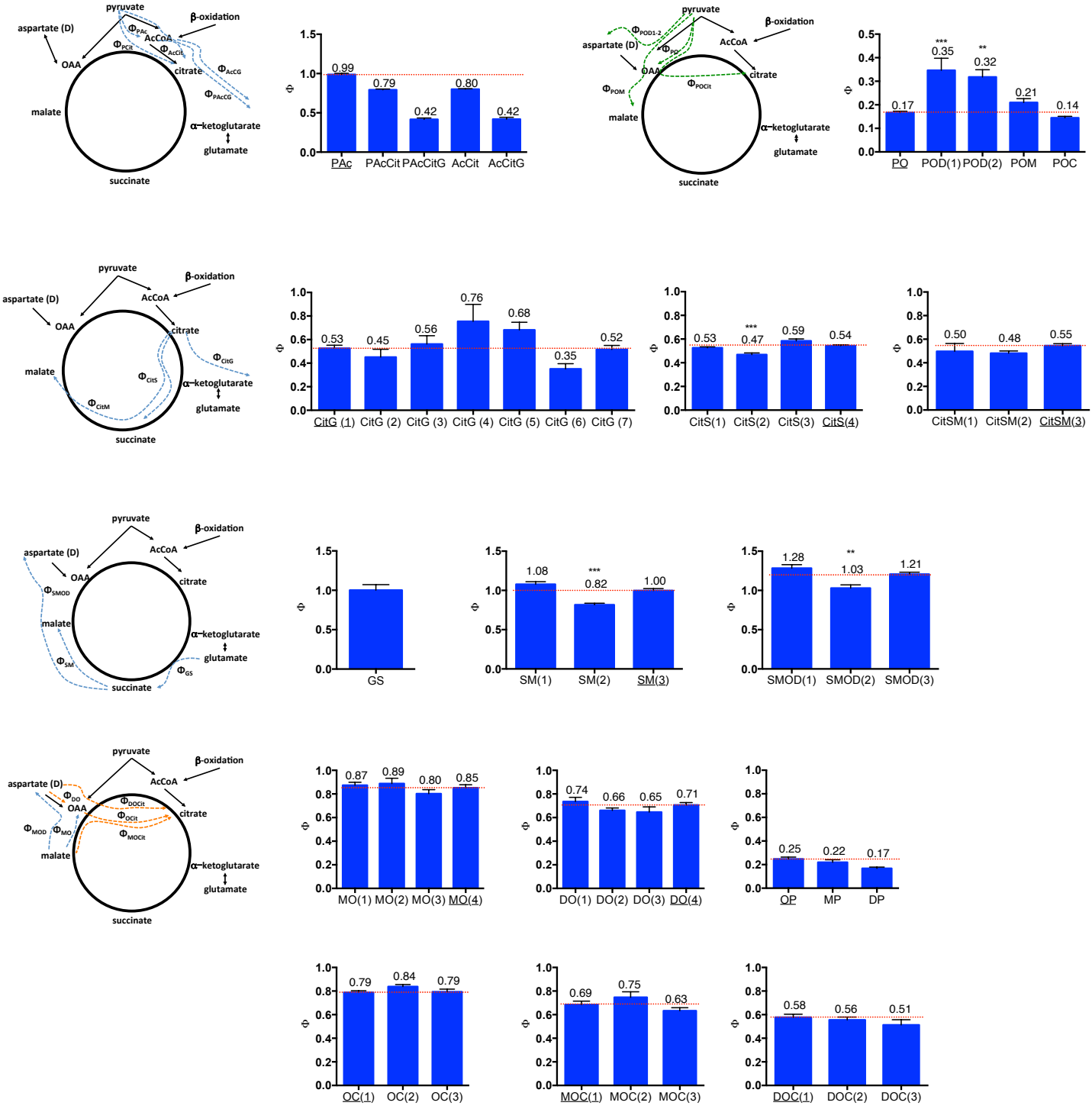
Supplemental References

Supplemental Figure S1 related to Figure 1 – Characterization of citrate isotopomers. (A) Parent/daughter distribution of the citrate fragments for M^{+2}, M^{+3}, M^{+4} and M^{+5}. (B) Citrate isotopomers generated by reverse ICDH.

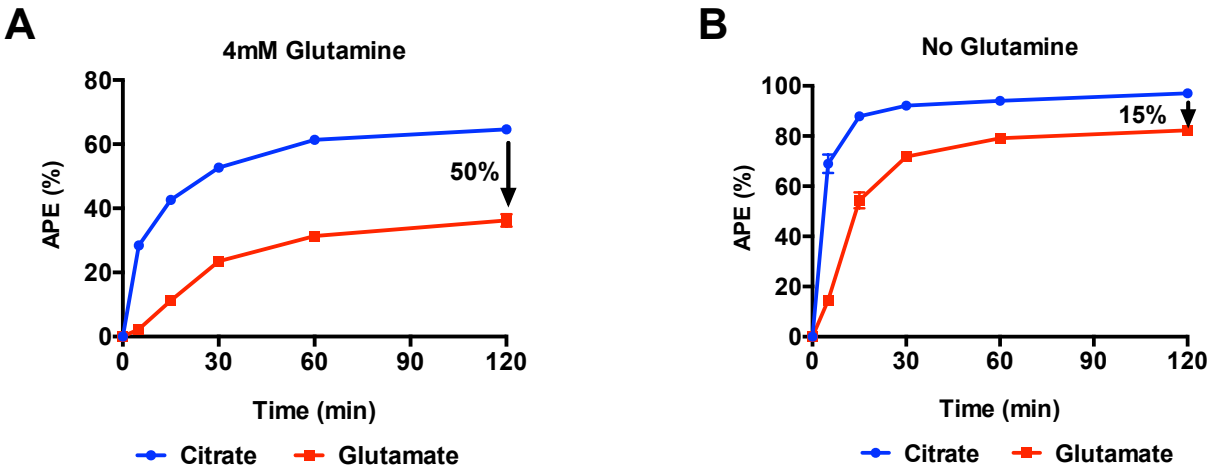




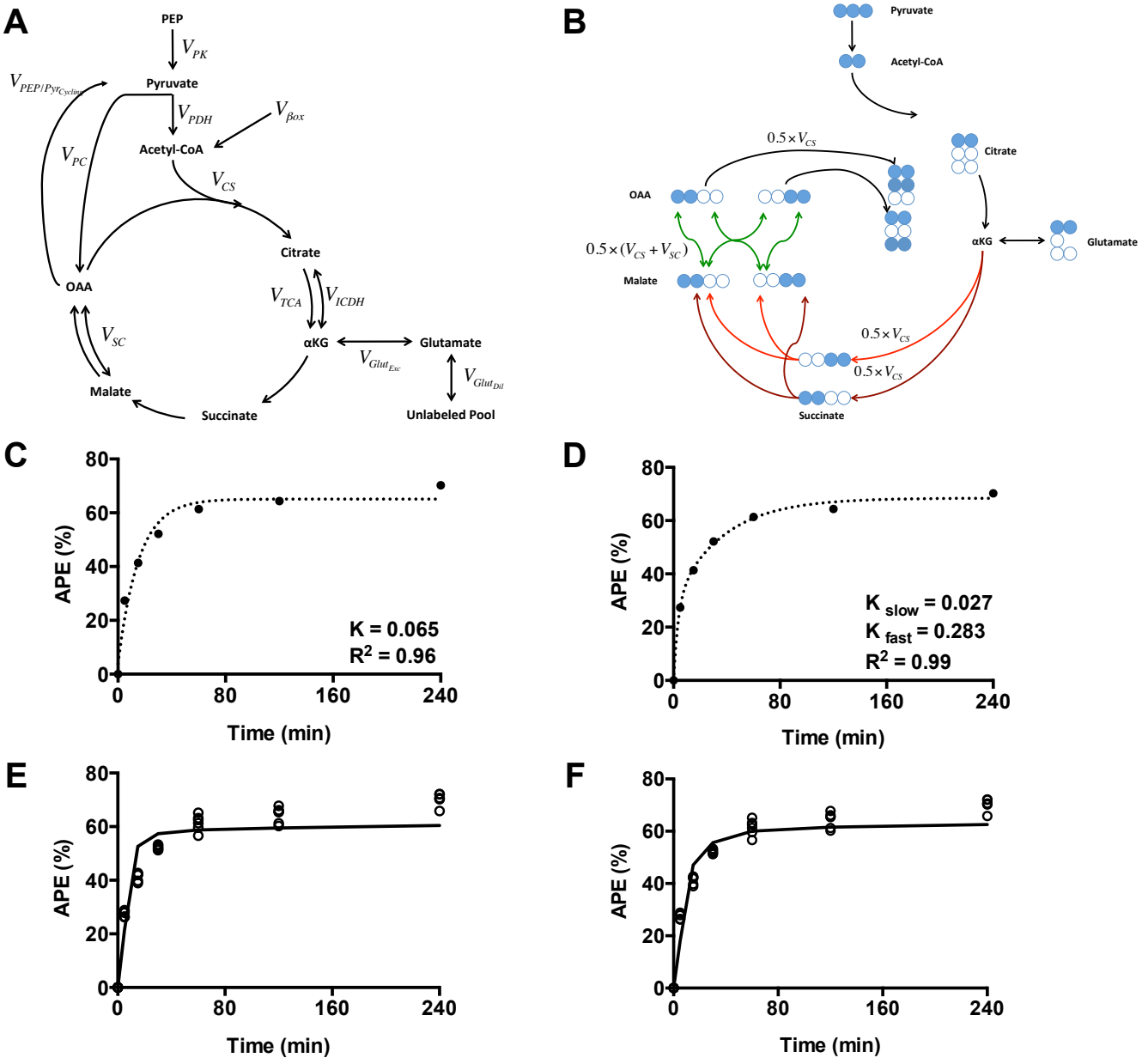
Supplemental Figure S2 related to Figure 3– Steady State (Φ) Analysis. Steady-state precursor-product relationships between the metabolic intermediates involved in the PC, PDH and TCA cycle reactions. The equations describing these relationships are shown in Supplemental Table S2. The (*) represents the relationship with the highest enrichments and therefore is used as a reference within a group of equivalent relationships. A red dashed line was drawn to facilitate that comparison.

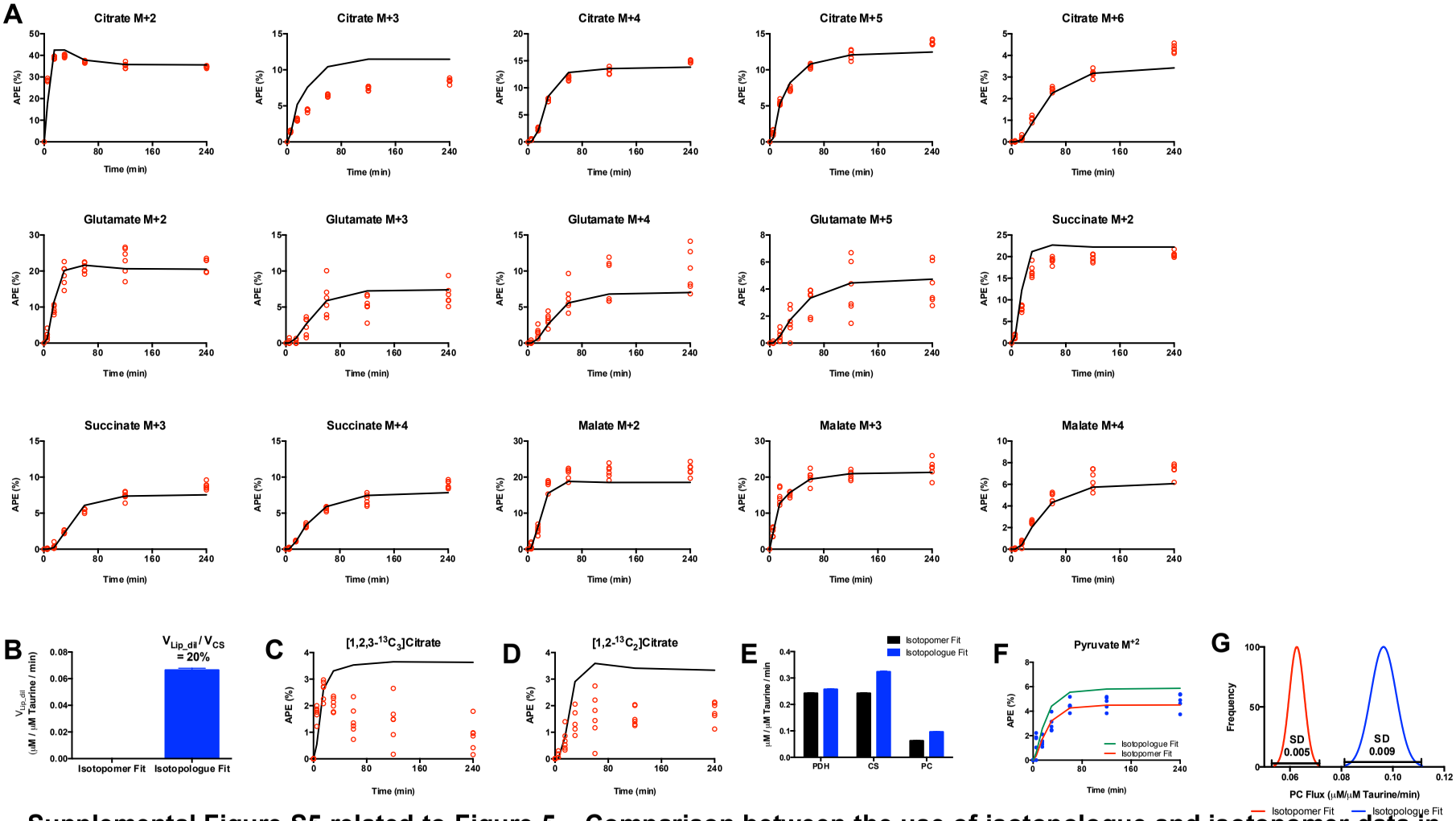


Supplemental Figure S3 Related to Figure 3– Impact of media glutamine on TCA labeling. Shown are (A) glutamate label dilution in the presence or (B) absence of glutamine. Both ^{13}C-enrichment curves refer to the label originated from the PDH reaction. Data are reported as mean ± S.E.M.



Supplemental Figure S4 Related to Figure 5– Description of the metabolic model using in CWave. (A) Schematic of the reactions included in the mathematical model used to determine citrate synthase (V_{CS}) flux. Pyruvate kinase (V_{PK}) is the reaction responsible for the enrichment of the pyruvate pool. ^{13}C-Label is then incorporated into the TCA cycle via pyruvate dehydrogenase (V_{PDH}) and pyruvate carboxylase (V_{PC}). PC flux is balanced by a rate converting oxaloacetate (OAA) into pyruvate ($V_{PEP/Pyr_{Cycling}}$). V_{ICDH} is the exchange between the citrate/isocitrate and the α-ketoglutarate (αKG) pools, catalyzed by isocitrate dehydrogenase. $V_{Glut_{Exc}}$ refers to the exchange reaction between αKG and glutamate catalyzed by the aspartate transaminase. $V_{Glut_{Dil}}$ is the reaction responsible by the dilution of glutamate. V_{SC} indicates the rate of exchange between the malate/fumarate pools and OAA responsible for the racemization of label. (B) Description of the label flow within the TCA cycle. (C) Difference between a single exponential and (D) a double (right) exponential fit describing the ^{13}C-citrate time course of ΣCit $_{a,f,i,d,h,j}$. (E) Modeled data with CWave assuming a simple exchange between citrate and an unlabeled source of carbons. (F) Modeled data with CWave assuming an exchange between citrate and αKG (V_{ICDH}). (Data are reported as mean ± S.E.M.





Supplemental Figure S5 related to Figure 5 – Comparison between the use of isotopologue and isotopomer data in the CWave model for flux calculation. (A) Fit curves of the isotopologue target data for citrate, glutamate, succinate and malate from INS-1 cells incubated with 9mM [U-$^{13}C_6$]glucose. The red open circles correspond to the enrichment data measured. The black lines correspond to the enrichments fit by the model. (B) $V_{\beta ox}$ calculated using the isotopomer and isotopologue data. (C-D) Examples of isotopomer fits that results from unlabeled acetyl-CoA: (C) [1,2,3-$^{13}C_3$]citrate and (D) [1,2-$^{13}C_2$]citrate. (E) PDH, PC and CS fluxes calculated using isotopomer and isotopologue data. (F) Prediction of M^{+2} pyruvate enrichments using the isotopomer (red line) and isotopologue data (green line). The blue circles correspond to the measured data. (G) PC flux values calculated by CWave using the isotopomer (red line) and isotopologue data (green line) and their respective standard deviation of the distribution.

Supplemental Figure S6 related to Figure 5 – **Fits of the time courses enrichments** of the isotopomers of citrate, glutamate, succinate, malate and OAA. The open circles correspond to the data were originated in INS-1 cells incubated with 9mM [U-$^{13}C_6$]glucose and analyzed by LC-MS/MS. The line indicates the enrichments predicted by the model analyzed with CWave. Overall LSSD: 1.39.

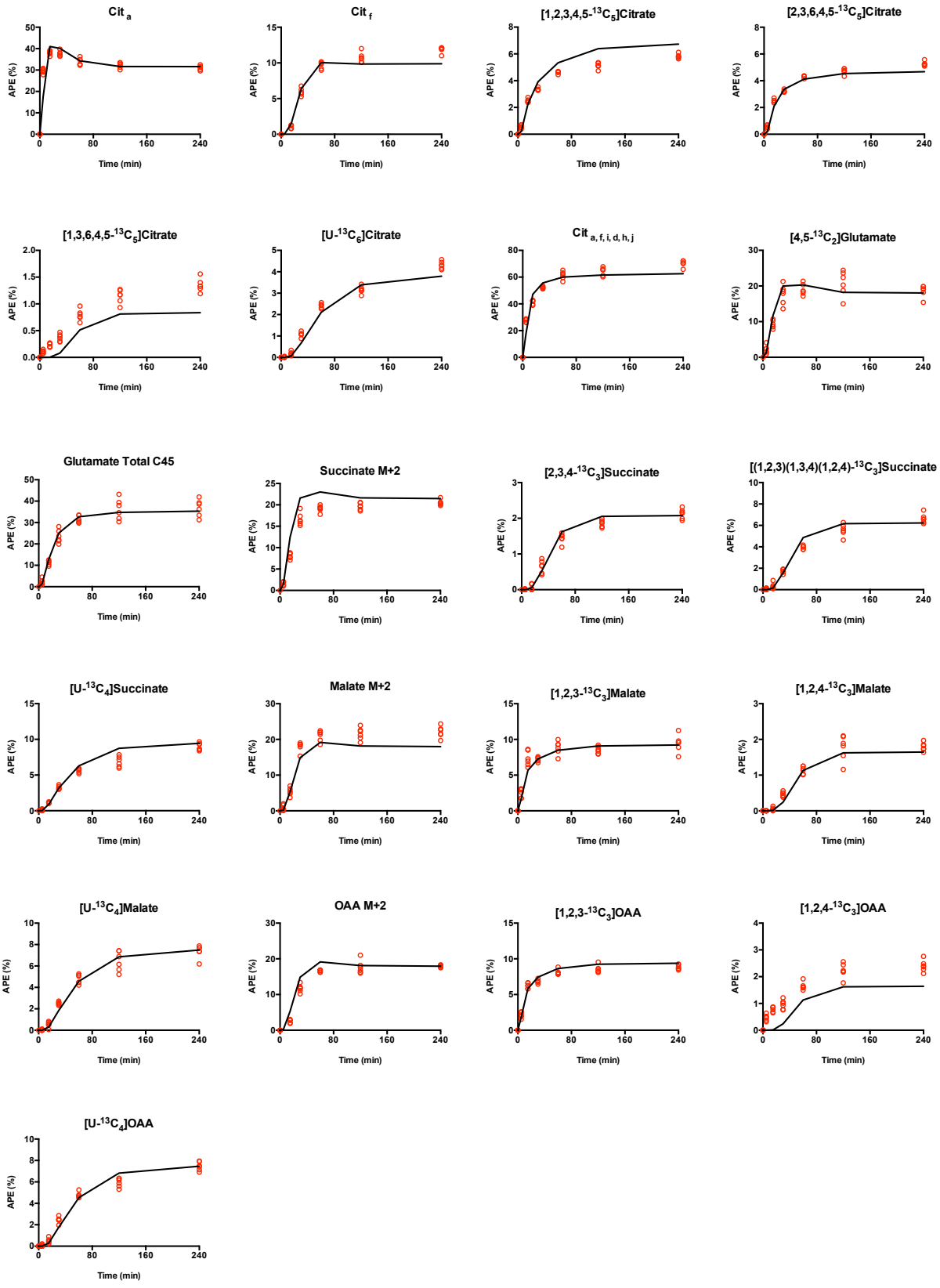


Figure S7 related to Methods – Isotopomer Analysis. Shown are the time-dependent ^{13}C-enrichment of succinate fragments (A) 118/73 and 118/74 (M^{+1}), (B) 119/74 and 119/75 (M^{+2}) and (C) 120/75 and 120/76 (M^{+3}). (D) Time-dependent ^{13}C-enrichment of malate isotopologues M^{+1}, M^{+2}, M^{+3} and M^{+4}. (E) Comparison of M^{+3} enrichments between malate and OAA. The M^{+3} enrichment was divided into two components: M^{+3} originated from the PC reaction and M^{+3} originated within the TCA cycle. (F) Time-dependent ^{13}C-enrichment of malate and aspartate M^{+2} are shown. (G) Comparison of the ^{13}C-enrichments from fragments 134/89 and 134/90 (aspartate M^{+2}). (H) Time-dependent ^{13}C-enrichment of the M^{+3} fragments, 135/90 and 135/91, from aspartate. (I) Comparison of aspartate M^{+3} from PC flux calculated with equations 22 and 25. All data are reported as mean ± S.E.M.

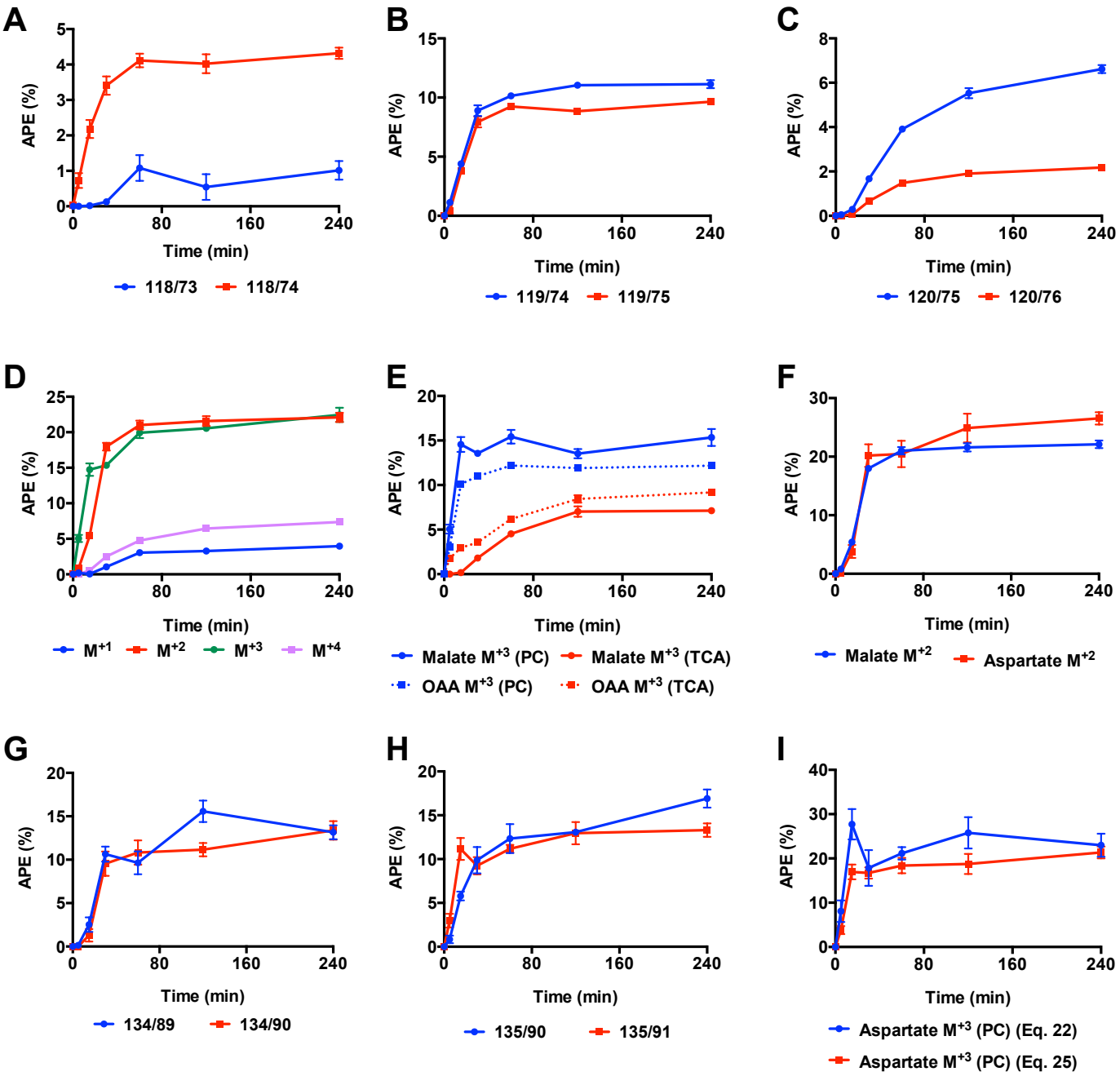


Table S1 Related to Figure 2– Calculation of Acetyl-CoA and OAA fractional enrichments based on citrate and glutamate isotopic data. Derivations in Supplemental Experimental Methods.

Acetyl-CoA Enrichments			
	$D_{A^*B^*}$	$S_{A\ B^*}$	
From Citrate Isotopomeric Families			
1	cit_f	cit_b	
2	cit_i	cit_e	
3	cit_h	cit_c	
4	cit_j	cit_g	
5	$\Sigma cit_{f,i,h,j}$	$\Sigma cit_{b,e,c,g}$	
From Glutamate Isotopomers			
6	*149/43*	*147/41*	
7	*150/43*	*148/41*	
8	*151/43*	*149/41*	
9	*149/43+150/43+151/43*	*147/41+148/41+149/41*	
OAA Enrichments			
	$D_{A^*B^*}$	S_{A^*B}	
From Citrate Isotopomeric Families			
10	[(1)(2)(3)(4)-$^{13}C_1$]OAA	cit_d	$\Sigma(cit_{a,f,h,i,j})$
11	[(1,2)(3,4)-$^{13}C_2$]OAA	cit_f	$\Sigma(cit_{a,d,h,i,j})$
12	[(1,2,3)(2,3,4)-$^{13}C_3$]OAA	cit_h	$\Sigma(cit_{a,d,f,i,j})$
13	[(1,2,3)(1,2,4)(1,3,4)(2,3,4)-$^{13}C_3$]OAA	cit_i	$\Sigma(cit_{a,d,f,h,j})$
14	[U-$^{13}C_4$]OAA	cit_j	$\Sigma(cit_{a,d,f,h,i})$

Supplemental Table S3 related to Figure 5: Combined pools of isotopomers defined in CWave and the correspondent target data.

Metabolite	Combined Isotopomers	Associated fragments or families of isotopomers
Citrate	[4,5-$^{13}C_2$]citrate	Cit_a
	[1,2,4,5-$^{13}C_4$], [3,6,4,5-$^{13}C_4$]citrate	Cit_f + ½ γ
	[1,2,3,4,5-$^{13}C_5$]citrate	½ Cit_h + ¼ Cit_i + α
	[2,3,6,4,5-$^{13}C_5$]citrate	½ Cit_h + ¼ Cit_i
	[1,3,6,4,5-$^{13}C_5$]citrate = [1,2,6,4,5-$^{13}C_5$]citrate	¼ Cit_i
	[1,2,3,6,4,5-$^{13}C_6$]citrate	Cit_j
Glutamate	[4,5-$^{13}C_2$]glutamate	148/43
	[4,5-$^{13}C_2$], [1,4,5-$^{13}C_3$], [2,4,5-$^{13}C_3$], [3,4,5-$^{13}C_3$], [1,2,4,5-$^{13}C_4$], [1,3,4,5-$^{13}C_4$], [2,3,4,5-$^{13}C_4$] and [1,2,3,4,5-$^{13}C_5$]glutamate	Σ(148/43, 149/43, 150/43, 151/43)
Succinate	[1,2-$^{13}C_2$], [3,4-$^{13}C_2$], [1,3-$^{13}C_2$], [1,4-$^{13}C_2$], [2,4-$^{13}C_2$] and [2,3-$^{13}C_2$]succinate	Σ(119/74, 119/75)
	[2,3,4-$^{13}C_3$]succinate	120/75
	[1,2,3-$^{13}C_3$], [1,3,4-$^{13}C_3$], [1,2,4-$^{13}C_3$]succinate	120/74
	[1,2,3,4-$^{13}C_4$]succinate	121/75
Malate	[1,2-$^{13}C_2$], [3,4-$^{13}C_2$], [1,3-$^{13}C_2$], [1,4-$^{13}C_2$], [2,4-$^{13}C_2$] and [2,3-$^{13}C_2$]malate	135/117
	[2,3,4-$^{13}C_3$], [1,2,3-$^{13}C_3$], [1,3,4-$^{13}C_3$], [1,2,4-$^{13}C_3$]malate	136/118
	[1,2,3,4-$^{13}C_4$]malate	137/119
OAA	[1,2-$^{13}C_2$], [3,4-$^{13}C_2$], [1,3-$^{13}C_2$], [1,4-$^{13}C_2$], [2,4-$^{13}C_2$] and [2,3-$^{13}C_2$]OAA	Total M+2
	[1,2,3-$^{13}C_3$]OAA = [2,3,4-$^{13}C_3$]OAA	½ (M^{+3} from PC) + ¼ (M^{+3} from TCA)
	[1,2,4-$^{13}C_3$]OAA = [1,3,4-$^{13}C_3$]OAA	¼ (M^{+3} from TCA)

Supplemental Table S4 related to Figure 5 – MIDA Analysis. Comparison between the steady state enrichments of Cit_f, Cit_h, Cit_i and Cit_j predicted by the enrichments of OAA and acetyl-CoA and those calculated using equations 7, 9, 10 and 11 from Supplemental Experimental Methods S2C.

	Predicted (%) (from AcCoA and OAA)		Measured (%)		Significance Student's T test	% Reduction Predicted vs. Measured		Significance 1-way ANOVA
Citrate Family	**Mean**	**SEM**	**Mean**	**SEM**	**P**	**Mean**	**SEM**	**P**
Cit_f	13.9	0.3	11.9	0.4	**0.0002**	14.5%	0.7%	**NS**
Cit_h	9.7	0.3	8.5	0.5	**0.0050**	12.3%	0.7%	
Cit_i	7.3	0.3	6.4	0.5	**0.0209**	13.2%	0.7%	
Cit_j	5.6	0.1	4.9	0.2	**0.0003**	11.9%	0.7%	
					Mean	13.0%		
					SEM	0.6%		

Supplemental Table S5 related to Figure 5: Metabolomic Analysis of INS-1 cells.
Concentrations (μM/μM Taurine) of PEP, pyruvate, citrate, glutamate, succinate, malate and aspartate as a function of glucose concentrations. Related to Figure 5. * P < 0.05, ** P < 0.0001 relative to G2.5 (one-way ANOVA).

Glucose	mM	2.5	5	7	9
PEP	mean	0.024	0.023	0.056	0.113
	SEM	0.001	0.002	0.004	0.011
	P			*	**
Pyruvate	mean	0.004	0.014	0.035	0.071
	SEM	0.001	0.001	0.002	0.005
	P			*	**
Citrate	mean	0.219	0.297	0.463	1.280
	SEM	0.012	0.018	0.034	0.071
	P			*	**
Glutamate	mean	3.949	4.263	5.090	7.633
	SEM	0.219	0.230	0.181	0.419
	P			*	**
Succinate	mean	0.020	0.015	0.018	0.040
	SEM	0.001	0.001	0.002	0.006
	P				*
Malate	mean	0.380	0.369	0.943	2.019
	SEM	0.038	0.043	0.073	0.283
	P			*	**
Aspartate	mean	7.025	4.573	4.073	2.745
	SEM	0.265	0.526	0.447	0.323
	P		*	**	**

Supplemental Table S6 related to table 1: Steady state enrichment analysis of INS-1 cells. Precursor/product analysis (APE/APE) of sequential reactions within the TCA cycle as a function of glucose concentrations. Graphical description of the different Φs found in Figure 3 and equations in Supplemental Table 2.

Glucose	ΦAcCit		ΦCitG		ΦGS		ΦSM		ΦMO		ΦOCit'		ΦOD	
mM	mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM
2.5	0.74	0.07	0.33	0.04	1.10	0.29	1.29	0.08	0.78	0.05	0.51	0.08	2.94	0.83
5	0.79	0.04	0.50	0.03	0.88	0.10	1.31	0.04	0.75	0.04	0.64	0.04	1.83	0.14
7	0.84	0.02	0.57	0.03	0.85	0.07	1.17	0.04	0.71	0.03	0.73	0.02	1.85	0.05
9	0.80	0.01	0.53	0.03	1.00	0.07	1.00	0.02	0.85	0.03	0.79	0.01	1.42	0.04

Supplemental Table S7 related to Methods- LC-MS/MS Analysis: MRM transition pairs (Q1/Q3), carbons analyzed in each fragment, mobile phase and approximate SV–CoV pair for each metabolite studied. M+n refers to the molecular weight (M) plus number of ^{13}C (n).

Metabolite	Q_1/Q_3		Daughter	Mobile Phase Composition	(SV, CoV)
Taurine	124/80		C1,2	15mM Ammonium Formate	(1400, -7.25)
PEP	M+0	167/79	C1,2,3	15mM Ammonium Formate	(1800, -13.25)
	M+1	168/79			
	M+2	169/79			
	M+3	170/79			
Pyruvate	M+0	87/87	C1,2,3	15mM Ammonium Formate	(1200, -4.5)
	M+1	88/88			
	M+2	89/89			
	M+3	90/90			
Malate	M+0	133/115	C1,2,3,4	15mM Ammonium Formate	(1800, -12.25)
	M+1	134/116			
	M+2	135/117			
	M+3	136/118			
	M+4	137/119			
Aspartate	M+0	132/88	C123 C234	15mM Ammonium Formate	(1800, -15.5)
	M+1	133/88			
		133/89			
	M+2	134/89			
		134/90			
	M+3	135/90			
		135/91			
	M+4	136/91			
Glutamate	M+0	146/41	C4,5	15mM Ammonium Formate	(1800, -12.5)
	M+1	147/41			
		147/42			
	M+2	148/41			
		148/42			
		148/43			
	M+3	149/41			
		149/42			
		149/43			
	M+4	150/42			
		150/43			
	M+5	151/43			
Citrate	M+0	191/67	C1,2,3,4 C2,3,4,5	15mM Ammonium Formate 0.1% Formic Acid 10uM EDTA	
	M+1	192/67			
		192/68			
	M+2	193/67			
		193/68			
		193/69			
	M+3	194/68			
		194/69			
		194/70			
	M+4	195/69			
		195/70			
		195/71			
	M+5	196/70			
		196/71			
	M+6	197/71			
Succinate	M+0	117/73	C123 C234	15mM Ammonium Formate 0.1% Formic Acid 10uM EDTA	
	M+1	118/73			
		118/74			
	M+2	119/74			
		119/75			
	M+3	120/75			
		120/76			
	M+4	121/76			

Supplemental Experimental Procedures

S1. ^{13}C-Isotopomer Labeling Studies

INS-1 cells were initially pre-incubated in DMEM medium (D5030, Sigma-Aldrich) supplemented with glucose (2.5, 5, 7 and 9mM), glutamine (4mM), pyruvate (0.05mM) and lactate (0.45mM) for 2h to reach metabolic steady state prior to the incorporation of label. Then they were washed with glucose-free DMEM medium and subsequently incubated with 2.5, 5, 7 and 9mM of [U-$^{13}C_6$]glucose (Cambridge Isotope Laboratories). Cells were quenched at different (n=6 per time point) by a rapid wash with ice-cold PBS and then collected in 150µl of a ice-cold solution containing 20% methanol, 0.1% formic acid, 1mM phenylalanine, 3mM NaF, 100uM EDTA and 10 µM $^{2}H_4$-taurine (CDN Isotopes) as a load control. All the samples were lyophilized and resuspended in 50µL of water prior the LC-MS/MS analysis.

S2A. LC-MS/MS Analysis

Samples were injected onto a Hypercarb column (3 µm particle size, 3x150 mm, Thermo Fisher Scientific) at a flow rate of 1 mL/min and separated isocratically. Samples were ionized by electrospray into an ABSCIEX 5500 QTRAP equipped with a SelexION for differential mobility separation (DMS) and acquired using multiple reaction monitoring (MRM) in negative mode. The source parameters were CUR: 30, CAD: high, IS: -1500, TEM: 625, GS1: 50 and GS2: 55. DMS parameters were DT: low, MD: 2-propanol, MDC: low, DMO: 3 and DR: off. Retention times were confirmed with known standards and peaks integrated using Multiquant (ABSCIEX) using the individual MRM transition pairs (Q_1/Q_3) and mobile phase composition for each metabolite (Supplemental Table S7). Fragment identities were confirmed using ^{13}C-labeled standards. DMS was used as an orthogonal separation axis for pyruvate and lactate, malate, aspartate, PEP, glyceraldehyde 3-phosphate and dihydroxyacetone phosphate to eliminate unknown isobaric contributions generated in the presence of mass label. Separation Voltage (SV) and Compensation Voltage (CoV) for each metabolite was optimized before each experiment. The atomic percent excess (APE) was calculated as the quotient between each Q_1/Q_3 area and the sum of all Q_1/Q_3 areas from each metabolite multiplied by 100. The values obtained from time 0 min were used to subtract background noise and adjust for natural abundance.

S2B. Natural Abundance Correction of MRM fragments

Natural abundance was adjusted based on the value of 1.1%. A corrected isotopomer matrix, $I'_{(P_m,D_n)}$, was generated to account for the presence of natural abundance carbons for each possible parent/daughter ion combination of the positive matrix $I_{(P_m,D_n)}$ (Eq 1).

$$I'_{(P_m,D_n)} = I_{(P_m,D_n)} * \big(1 + k(p-m)\big) - I_{(P_{m-1},D_n)} * k\big((p-d) - (m-n-1)\big) - I_{(P_{m-1},D_{n-1})} * k\big(d - (n-1)\big) \quad \text{(Eq 1)}$$

In equation 1:

- p is the total number of carbons in the parent ion
- d is the total number of carbons in the daughter ion
- m is the number of ^{13}C in the parent ion
- n is the number of ^{13}C in the daughter ion
- I is the peak area corresponding to parent ion with mass P from $0 \rightarrow p$ and daughter ion with mass D from $0 \rightarrow d$
- k is 0.011 (the natural abundance of ^{13}C in the environment)
- $m - n \leq p - d$

S2C. Deconvolution of Citrate Isotopomers

Citrate is a symmetric molecule but it contains a prochiral center that can be stereochemically distinguished by enzymes of the TCA cycle. The well-established recognition of positional transfer of labeled carbons from metabolite to metabolite in the TCA cycle forms the basis of these flux experiments. Rather than using standard prochiral nomenclature to identify the carbons, citrate carbons are numbered as shown in Figure 1A based on the standard numbering of glutamate carbons. This allows easier comparison of the positional transfer of labeled carbons from those metabolites with prochirality (e.g., citrate, fumarate) to those without (e.g., succinate, malate, glutamate). Acetyl-CoA generated via β-oxidation or the PDH reaction contributes carbons 4 and 5 (per this numbering scheme) while those derived from OAA provide carbons 1, 2, 3 and 6. The metabolism of [U-$^{13}C_6$]glucose will generate [U-

$^{13}C_3$]pyruvate that then enters the TCA cycle via either PDH (dark blue) or PC (dark green). As these labeled pyruvate carbons flow through subsequent turns of the TCA cycle, additional labeling patterns are generated (light blue and green). All of the isotopomers generated by ^{13}C carbons coming from PDH flux can be measured if all of the isotopomers with ^{13}C in positions 4,5 (citrate isotopomer families $cit_{a,d,f,h,i,j}$ indicated in dark blue) can be measured. Similarly, PC-derived carbons are represented by isotopomer families $cit_{c,h}$ *(dark green).* The division of the citrate isotopomers into families is based on the condensation of OAA with labeled or unlabeled acetyl-CoA. Within each family all the isotopomers have equal probability (assumption 5). These stoichiometric relationships make it possible to fully deconvolve citrate isotopomers.

Citrate prochirality, while recognizable by enzymes, does not alter fragmentation patterns. Any asymmetric fragmentation of a citrate parent ion has equal probability of generating of pro-S and pro-R daughters. The ***191/67*** citrate parent/daughter anion occurs from the loss of carbons C1,6 and with equal probability carbons C5,6. The fragmentation patterns of the citrate mass isotopomer families generated from uniformly labeled pyruvate are shown in Supplemental Figure S1-A.

The assignments of the parent/daughter combinations for the individual M^{+2} to M^{+6} mass isotopomer families is described below:

Citrate M^{+2}: There are three ways to generate M^{+2} labeling of citrate: from PDH to generate cit_a as well as from second cycle TCA from either PDH or PC flux to generate the indistinguishable families cit_b and $cit_{b'}$. Because they do not provide unique flux information, for simplicity they will be collapsed into the single term cit_b representing the combination (Figure 1A). Fragmentation of family cit_a will generate equal amounts of ***193/68*** and ***193/69*** daughters. Fragmentation of cit_b generates three ***193/68*** daughters for every one ***193/69***

daughter. Therefore, the excess ***193/68*** is attributable to family cit_b. Based on the distribution of the fragments (Supplemental Figure S1-A) we can derive the equations describing the isotopomer composition of each Q_1/Q_3 parent daughter combination:

$$\mathbf{193/68} = \frac{a}{2} + \frac{3b}{4}$$

$$\mathbf{193/69} = \frac{a}{2} + \frac{b}{4}$$

where ***a*** represents the isotopomers from labeled acetyl-CoA with [4,5-$^{13}C_2$] labeled, and where ***b*** is the combination of [3,6-$^{13}C_2$] and [1,2-$^{13}C_2$] both arising from second cycle (from either PDH or PC flux). From assumption 5, the enrichments of [1,2-$^{13}C_2$]citrate and [3,6-$^{13}C_2$]citrate are equal and reduce to a single term represented as [**Σ**(1,2)(3,6)-$^{13}C_2$]citrate. The enrichments of the possible isotopomers considered for M^{+2} are calculated according to equations 2 and 3 after solving for ***a*** and ***b***, respectively.

$$cit_a = \left[4{,}5 - {}^{13}C_2\right]Citrate = 3 * \mathbf{193/69} - \mathbf{193/68} \qquad \text{(Eq 2)}$$

$$cit_b = \left[\Sigma(3{,}6)(1{,}2) - {}^{13}C_2\right]Citrate = 2 * (\mathbf{193/68} - \mathbf{193/69}) \qquad \text{(Eq 3)}$$

Citrate M^{+3}: Three groups of citrate M^{+3} isotopomers come from PC (cit_c), *PDH M^{+2} plus M^{+1} OAA* (cit_d), and the four possible M^{+3} OAA patterns coming from second cycle of cit_f *(family* cit_e). Based on the distribution of the fragments we can derive the equations describing the isotopomer composition of each Q_1/Q_3 parent daughter combination:

$$\mathbf{194/68} = \frac{1}{8}d + \frac{2}{8}e$$

$$\mathbf{194/69} = \frac{3}{4}c + \frac{5}{8}d + \frac{5}{8}e$$

$$\mathbf{194/70} = \frac{1}{4}c + \frac{2}{8}d + \frac{1}{8}e$$

The enrichments of all the isotopomers considered for M^{+3} are calculated according to equations 4-6 (after solving for ***c***, ***d*** and ***e***, respectively).

$$cit_c = \left[\Sigma(1,2,3)(2,3,6) - {}^{13}C_3\right]Citrate = 3 * \mathbf{194/69} - 5 * \mathbf{194/68} - 5 * \mathbf{194/70} \quad \text{(Eq 4)}$$

$$cit_d = \left[\Sigma(1,4,5)(2,4,5)(3,4,5)(6,4,5) - {}^{13}C_3\right]Citrate = 4 * \mathbf{194/68} - 4 * \mathbf{194/69} + 12 * \mathbf{194/70} \quad \text{(Eq 5)}$$

$$cit_e = \left[\Sigma(1,2,3)(2,3,6)(1,2,6)(1,3,6) - {}^{13}C_3\right]Citrate = 2 * \mathbf{194/68} + 2 * \mathbf{194/69} - 6 * \mathbf{194/70} \quad \text{(Eq 6)}$$

Citrate M^{+4}: There are two M^{+4} isotopomer families. The first, cit_f, comes from [1,2-$^{13}C_2$]Acetyl-CoA from PDH flux combined with the second turn of the TCA cycle carrying forward the condensation of OAA labeled from PDH. Alternatively, the same labeling pattern is generated by first cycle PDH combined with second cycle PC ($cit_{f'}$). The families cit_f and $cit_{f'}$ are indistinguishable and therefore collapsed into a single isotopomer family (cit_f). Family cit_g arises during the second turn of the TCA cycle from the condensation of M^{+4} OAA coming

(from cit_h) with unlabeled acetyl-CoA. Based on the distribution of the fragments we can derive the equations describing the isotopomer composition of each Q_1/Q_3 parent daughter combination:

$$\mathbf{196/69} = \frac{1}{4}f + \frac{1}{2}g$$

$$\mathbf{195/70} = \frac{3}{4}f + \frac{1}{2}g$$

The enrichments of all the isotopomers considered for M^{+4} are calculated according to equations 7 and 8 after solving for ***f*** and ***g***, respectively.

$$cit_f = \left[\Sigma(1,2,4,5)(3,6,4,5) - {}^{13}C_4\right]Citrate = 2 * \mathbf{195/70} - 2 * \mathbf{195/69} \quad \text{(Eq 7)}$$

$$cit_g = \left[1,2,3,6 - {}^{13}C_4\right]Citrate = 3 * \mathbf{195/69} - \mathbf{195/70} \quad \text{(Eq 8)}$$

Citrate M^{+5}: There are two families of M^{+5} isotopomers. Family cit_h results from the combination of PC and PDH fluxes during the first turn of the TCA cycle. Family cit_i arises from labeled PDH flux plus the recycling of family cit_f during the third turn of the TCA cycle. Based on the distribution of the fragments the equations describing the isotopomer composition of each Q_1/Q_3 are:

$$\mathbf{196/70} = \frac{1}{4}h + \frac{5}{8}i$$

$$\mathbf{196/71} = \frac{3}{4}h + \frac{3}{8}i$$

The enrichments of all the isotopomers considered for M^{+5} are calculated according to equations 9 and 10 after solving for ***h*** and ***i***, respectively.

$$cit_h = \left[\Sigma(1,2,3,4,5)(2,3,6,4,5) - {}^{13}C_5\right]Citrate = \frac{5}{3} * \mathbf{196/71} - \mathbf{196/70} \quad \text{(Eq 9)}$$

$$cit_i = \left[\Sigma(1,2,3,4,5)(2,3,6,4,5)(1,3,6,4,5)(1,2,6,4,5) - {}^{13}C_5\right]Citrate = 2 * \mathbf{196/70} - \frac{2}{3} * \mathbf{196/71} \quad \text{(Eq 10)}$$

Citrate M^{+6}: This family contains a single member formed from the condensation of OAA derived from cit_h with [1,2-$^{13}C_2$]Acetyl-CoA. It gives a unique fragmentation pattern (***197/71***):

$$cit_j = \left[1,2,3,4,5,6 - {}^{13}C_6\right]Citrate = \mathbf{197/71} \quad \text{(Eq 11)}$$

S2D. Correction for Isocitrate Dehydrogenase Exchange

This deconvolution assumes a direct flow of carbons from citrate to aKG. However, the existence of a reversed flow through ICDH creates an exchange between citrate and aKG. The practical implication is that the reversed flux through ICDH will impact the labeling patterns of citrate (Supplemental Figure S1-B). Because bicarbonate is assumed to not contribute significantly to the labeling patterns, the reverse flux through ICDH will only affect citrate isotopomers containing ^{13}C in position 6. For instance, the decarboxylation of [U-$^{13}C_6$]citrate yields [1,2,3,4,5-$^{13}C_5$]αKG, which through reverse ICDH generates [1,2,3,4,5-$^{13}C_5$]citrate that was not originated by [1,2,3-$^{13}C_3$]OAA. Because the label in carbons 4 and 5 is not affected by

reverse ICDH, the relevance of this flux is proportional to the ratio between the sum of all [4,5-^{13}C] isotopomers (Σ Cit_a, Cit_f, Cit_i, Cit_h, Cit_d, Cit_j) and [1,2-$^{13}C_2$]acetyl-CoA, defined as Φ_{AcCit} (Isotopic Steady-State Relationships). The following equations use Φ_{AcCit} to correct the area of each Q1/Q3 citrate fragments in order to account for the reverse ICDH flux:

M^{+6}

$$\mathbf{197/71_c} = \frac{\mathbf{197/71}}{\boldsymbol{\Phi_{AcCit}}}$$

M^{+5}

$$\mathbf{196/71_c} = \mathbf{196/71} - \mathbf{197/71}(1 - \Phi_{AcCit}) + \mathbf{195/71}$$

$$\mathbf{196/70_c} = \frac{\mathbf{196/70}}{\Phi_{AcCit}}$$

M^{+4}

$$\mathbf{195/70_c} = \mathbf{195/70} - \mathbf{196/70}(1 - \Phi_{AcCit}) + \mathbf{195/69}(1 - \Phi_{AcCit})$$

$$\mathbf{195/69_c} = \frac{\mathbf{195/69}}{\Phi_{AcCit}}$$

M^{+3}

$$\mathbf{194/70_c} = \mathbf{194/70} - \mathbf{195/69}(1 - \Phi_{AcCit})$$

$$\mathbf{194/69_c} = \mathbf{194/69} - \mathbf{195/69}(1 - \Phi_{AcCit}) + \mathbf{194/68}(1 - \Phi_{AcCit}) + \mathbf{193/67}$$

$$\mathbf{194/68_c} = \frac{\mathbf{194/68}}{\Phi_{AcCit}}$$

M^{+2}

$$\mathbf{193/69_c} = \mathbf{193/69 - 194/68}(1 - \Phi_{AcCit})$$

$$\mathbf{193/68_c} = \mathbf{193/68 - 194/68}(1 - \Phi_{AcCit}) + (\mathbf{193/68 - 193/69})(1 - \Phi_{AcCit})$$

S2E. Deconvolution of glutamate isotopomers.

Further metabolism of citrate to αKG in the TCA cycle results in loss of C6 of citrate but C1-C5 retain their positional labeling. αKG is in rapid exchange (via reversible transamination or via anaplerotic entry via GDH) with glutamate – a highly concentrated and commonly used surrogate of the TCA cycle.

Carbons 4 and 5 coming from acetyl-CoA are directly measured in the 2-carbon ***146/41*** daughter. This fragment effectively divides glutamate into its two precursor components: acetyl-CoA (glutamate C4 and C5) and OAA (glutamate C1-C3). Consequently, all of the glutamate isotopomers with daughters of ***41*** have no enrichment ($\boldsymbol{Glu_{C4,5^0}}$), with daughters of ***42*** are M^{+1} enriched ($\boldsymbol{Glu_{C4,5^{+1}}}$), and ***43*** are M^{+2} enriched ($\boldsymbol{Glu_{C4,5^{+2}}}$). It is not possible to determine the position of the enriched carbon in $\boldsymbol{Glu_{C4,5^{+1}}}$.

Because of the lack of additional positional enrichment in carbons 1-3 from the fragmentation, only the isotopologues of this fragment can be assessed (M, M^{+1}, M^{+2}, M^{+3}). While there is no unique solution for the M^{+1} and M^{+2} isotopologues of the C1-3 glutamate fragment, [U-$^{13}C_4$]OAA and [2,3,4 $^{13}C_3$]OAA (but not [1,2,3 $^{13}C_3$]OAA) are the precursors for the M^{+3} labeled C1-3 of glutamate.

Glutamate C4,5 isotopologues:

$Glu_{C4,5^0}$ = [C4,5 $^{13}C_0$]glutamate = **Σ(*146/41, 147/41, 148/41*, *149/41)*** (Eq 12)

$Glu_{C4,5^{+1}}$ = [C4,5 $^{13}C_1$]glutamate = **Σ(*147/42, 148/42, 149/42*,*150/42)*** (Eq 13)

$Glu_{C4,5^{+2}}$ = [C4,5 $^{13}C_2$]glutamate = **Σ(*148/43, 149/43, 150/43*, *151/43)*** (Eq 14)

Glutamate C1-3 isotopologues

$Glu_{C1,2,3^0}$ = [C1,2,3 $^{13}C_0$]glutamate = **Σ(*146/41, 147/42, 148/43)*** (Eq 15)

$Glu_{C1,2,3^{+1}}$ = [C1,2,3 $^{13}C_1$]glutamate = **Σ(*147/41, 148/42, 149/43)*** (Eq 16)

$Glu_{C1,2,3^{+2}}$ = [C1,2,3 $^{13}C_2$]glutamate = **Σ(*148/41, 149/42, 150/43)*** (Eq 17)

$Glu_{C1,2,3^{+3}}$ = [C1,2,3 $^{13}C_3$]glutamate = **Σ(*149/41, 150/42, 151/43)*** (Eq 18)

The present inability to discern positional labeling within the daughter ion containing C1-3 limits the possibility of fully deconvolving this metabolite.

S2F. Deconvolution of Succinate isotopomers

Succinate is an excellent readout of TCA cycle activity. It is formed almost exclusively in the mitochondria. Unlike malate and fumarate, the labeling pattern of succinate is not directly affected by PC flux since succinic dehydrogenase (SDH) is unidirectional and doesn't receive carbons from fumarate under normal conditions. Therefore, succinate labeling is a consequence of label passing through CS. Since succinate is symmetric, ^{13}C-label is evenly distributed across the molecule. The ***117/73*** fragment corresponds to the loss of either C1 or C4 (Supplemental Table S7) and can be used to obtain the positional enrichment of ^{13}C-label:

Succinate M^{+1}

There is an equal probability of M^{+1} enrichment in all four carbons. The relationship 3 x ***118/74*** = ***118/73*** is predicted based on the labeling scheme was observed (Supplemental Figure S7-A).

Succinate M^{+2}

Doubly labeled succinate will be equally label C1,2 and C3,4 with other combinations not likely. Here, ***119/74*** closely approximates ***119/75*** at all times (Supplemental Figure S7-B).

Succinate M^{+3}

There are four possible labeling patterns for M^{+3} succinate: [(1,2,3)(2,3,4)(1,3,4)(1,2,4)-$^{13}C_3$]. These four isotopomers arise from Cit_i and thus have equal probability of occurring. For this reason, the predicted relationship **120/75 = 120/76** x 3 is confirmed and rules out the contribution of meaningful reverse labeling of succinate (Supplemental Figure S7-C).

Succinate M^{+4}

There is only one possible fragmentation pattern: ***120/76***

S2G. Deconvolution of Malate Isotopomers

The fragment used to study malate is the result of a dehydrogenation and contains all four carbons (Supplemental Table S7) and thus positional enrichments cannot be resolved. Supplemental Figure S7-D shows the time course for four possible isotopologues.

Positional enrichments for M^{+3} are needed to distinguish $\left[(1,2,3)(2,3,4) - {}^{13}C_3\right]malate$ generated through PC, from $\left[(1,2,3)(2,3,4)(1,2,4)(1,3,4) - {}^{13}C_3\right]malate$ generated within the TCA cycle. The isotopomers, however, can be deduced considering the irreversibility of SDH. Since there is no direct PC generated M^{+3} label contribution to succinate then malate can be corrected by a weighted subtraction of succinate enrichments: $\left[(1,2,3)(2,3,4)(1,2,4)(1,3,4) - {}^{13}C_3\right]malate$ is predicted based from $\left[(1,2,3)(2,3,4)(1,2,4)(1,3,4) - {}^{13}C_3\right]succinate$ (Eq 19). In this calculation, the dilution between the malate and succinate pools must be accounted for. Because $\left[U - {}^{13}C_4\right]malate$ can only be generated from $\left[U - {}^{13}C_4\right]succinate$, the ratio $\left[U - {}^{13}C_4\right]malate/\left[U - {}^{13}C_4\right]succinate$ can be used as a correction factor for that dilution. The enrichments originating from the PC reaction, $\left[(1,2,3)(2,3,4) - {}^{13}C_3\right]malate$, can then be obtained by subtraction from the total M^{+3} enrichments (Eq 20). The malate enrichments obtained from equations 19 and 20 are very similar to the OAA enrichments (Supplemental Figure S7-E).

$$\left[(1,2,3)(2,3,4)(1,2,4)(1,3,4) - {}^{13}C_3\right]malate = \left[(1,2,3)(2,3,4)(1,2,4)(1,3,4) - {}^{13}C_3\right]succinate * \frac{\left[U-{}^{13}C_4\right]malate}{\left[U-{}^{13}C_4\right]succinate} \quad \text{(Eq 19)}$$

$$\left[(1,2,3)(2,3,4) - {}^{13}C_3\right]malate = \left[{}^{13}C_3\right]malate - \left[(1,2,3)(2,3,4)(1,2,4)(1,3,4) - {}^{13}C_3\right]malate \quad \text{(Eq 20)}$$

S2H. Deconvolution of Aspartate isotopomers

De novo synthesis of OAA from pyruvate via PC incorporates natural abundance CO_2 into C4 in an open system (Assumption 2). If OAA is in near-equilibrium with malate across fumarase, this racemically scrambles this carbon between positions 1 and 4 (and carbon 2 with 3). Since aspartate and malate share OAA as a common intermediate, the equivalence of aspartate and malate M^{+2} enrichments, arising from the turning of the TCA cycle, suggest these pools are in isotopic equilibrium with each other through OAA (Supplemental Figure S7-F). The asymmetric fragmentation of aspartate leads to loss of C4 (Supplemental Table S7) thus allowing positional assignment of label.

Aspartate M^{+1}

There is no advantage to deconvolving these isotopomers since there is an equal probability of M^{+1} enrichment in all four carbons. Nevertheless, the relationship 3 x ***133/88*** = ***133/89*** is predicted based on the labeling scheme.

Aspartate M^{+2}

Doubly labeled aspartate will be equally C1,2 and C3,4 with other combinations not likely. Here, ***134/89*** equals ***134/90*** confirming the expected symmetry (Supplemental Figure S7-G)**.**

Aspartate M^{+3}

As with malate, there are two possible labeling patterns for M^{+3} aspartate. The [(1,2,3)(2,3,4)-$^{13}C_3$] arising from PC flux and [(1,2,3)(1,2,4)(1,3,4)(2,3,4)-$^{13}C_3$] generated within the TCA cycle. The former would generate equal amounts of ***135/90*** and ***135/91***. The latter, would generate three ***135/90*** for every ***135/91***. Supplemental figure S7-H shows the time

courses for both ***135/90*** and ***135/91***. The amount coming from TCA, PC, and total M^{+3} then are defined by the following

$\left[(1,2,3)(2,3,4)(1,2,4)(1,3,4) - {}^{13}C_3\right]aspartate$ = 2 * (**135/90**-**135/91**) (Eq. 21)

$\left[(1,2,3)(2,3,4) - {}^{13}C_3\right]aspartate$ = 3*(**135/91**)- **(135/90)** (Eq. 22)

$\left[{}^{13}C_3\right]aspartate$ = (**135/90**+**135/91**) (Eq. 23)

Alternatively, $\left[(1,2,3)(2,3,4) - {}^{13}C_3\right]aspartate$ can also be determined using the same principle described above to calculate malate M^{+3} isotopomers. Therefore, $\left[(1,2,3)(2,3,4)(1,2,4)(1,3,4) - {}^{13}C_3\right]$ and $\left[(1,2,3)(2,3,4) - {}^{13}C_3\right]aspartate$ were calculated according to equations 24 and 25. Both approaches yield similar results (Supplemental Figure S7-I).

$$\left[(1,2,3)(2,3,4)(1,2,4)(1,3,4) - {}^{13}C_3\right]aspartate = \left[(1,2,3)(2,3,4)(1,2,4)(1,3,4) - {}^{13}C_3\right]succinate * \frac{\left[U-{}^{13}C_4\right]aspartate}{\left[U-{}^{13}C_4\right]succinate} \quad \text{(Eq 24)}$$

$$\left[(1,2,3)(2,3,4) - {}^{13}C_3\right]aspartate =$$

$$\left[{}^{13}C_3\right]aspartate - \left[(1,2,3)(2,3,4)(1,2,4)(1,3,4) - {}^{13}C_3\right]aspartate \quad \text{(Eq 25)}$$

Aspartate M^{+4}

There is only one possible fragmentation pattern: ***136/91***

S3. Calculation of acetyl-CoA and OAA enrichments (MIDA)

The relative contribution of glucose oxidation and β-oxidation to the acetyl-CoA used by citrate synthase can be determined if both pyruvate and acetyl-CoA enrichments are known. While in practice, directly measuring the fractional enrichment of the mitochondrial matrix pool of acetyl-CoA and OAA can be a challenge; nevertheless, it is solvable by mass isotopomer distribution analysis (MIDA) (Hellerstein and Neese, 1992).

In a reaction where two substrates (A and B) combine to form a product (AB), if both substrates are fractionally enriched (FE_{A^*} and FE_{B^*}), then MIDA determines the enrichment of both precursors even if there is dilution from outside unlabeled product. For the reaction A + B → AB the fractional enrichment of the substrates are defined as

$$FE_{A^*} = \left(\frac{A^*}{A+A^*}\right) \quad \text{(Eq. 26)}$$

$$FE_{B^*} = \left(\frac{B^*}{B+B^*}\right) \quad \text{(Eq. 27)}$$

where (*) designates the presence of a measurable label.

The reaction of (A + A*) with (B + B*) will generate the populations of

$$AB + A^*B + AB^* + A^*B^* = 1 \quad \text{(Eq. 28)}$$

by their binomial distribution even if there is a contaminating source of unlabeled product (A'B') as described by Eq 29.

$$AB + A^*B + AB^* + A^*B^* + A'B' = 1 \quad \text{(Eq. 29)}$$

The probability of generating the doubly labeled product ($D_{A^*B^*}$) and singly labeled substrates (S_{A^*B} and $S_{A\ B^*}$) are determined by the fractional enrichments in the compartment where the product is formed:

$$D_{A^*B^*} = \left(\frac{A^*}{A+A^*}\right) * \left(\frac{B^*}{B+B^*}\right) \quad \text{(Eq. 30)}$$

$$S_{A^*B} = \left(\frac{A^*}{A+A^*}\right) * \left(\frac{B}{B+B^*}\right) \quad \text{(Eq. 31)}$$

$$S_{A\ B^*} = \left(\frac{A}{A+A^*}\right) * \left(\frac{B^*}{B+B^*}\right) \quad \text{(Eq. 32)}$$

The ratio of singly to doubly labeled products is described by equations 33 and 34.

$$\frac{S_{A\ B^*}}{D_{A^*B^*}} = \frac{A}{A^*} \quad \text{(Eq. 33)}$$

$$\frac{S_{A^*B}}{D_{A^*B^*}} = \frac{B}{B^*} \quad \text{(Eq. 34)}$$

Solving equations 33 and 34 for A and B and then substituting into the equations for the fractional enrichments the FE_{A^*} and FE_{B^*} (Eq 26 and 27) are determined:

$$FE_{A^*} = \frac{1}{\frac{S_{A\ B^*}}{D_{A^*B^*}}+1} \quad \text{(Eq. 35)}$$

$$FE_{B^*} = \frac{1}{\frac{S_{A^*B}}{D_{A^*B^*}}+1} \quad \text{(Eq. 36)}$$

Citrate (and subsequently glutamate) is formed in the mitochondrial matrix via citrate synthase through the condensation acetyl-CoA and OAA. Unlike NMR, mass spectroscopy evaluates individual molecules. Therefore, it is possible determine $D_{A^*B^*}$, $S_{A\ \ B^*}$, and S_{A^*B} from isotopomer deconvolution (see Deconvolution of Citrate Isotopomers). There are several potential ways to calculate the fractional enrichments of both acetyl-CoA (FE_{A^*}) and OAA (FE_{B^*}) in the mitochondrial matrix. Supplemental Table S1 shows a list of all the equivalent alternatives to calculate [1,2-$^{13}C_2$]acetyl-CoA from citrate and glutamate isotopomer analysis.

In practice, options (5) and (9) provide the best possible signal/noise and were used to calculate [1,2-$^{13}C_2$]acetyl-CoA from citrate and from glutamate. Of note, they are similar at steady state (Citrate 80±2% vs. Glutamate 86±2%, N.S.). MIDA can also be applied to calculate the mitochondrial enrichments of OAA. Since one OAA carbon is lost in the conversion of citrate into glutamate, then it is not possible to calculate the relative OAA isotopomers from glutamate (Supplemental Table S1).

Together, the calculated steady-state enrichments of OAA and acetyl-CoA can be used to predict the enrichments of several citrate isotopomers. Any given citrate isotopomer is the result of the product between OAA and acetyl-CoA enrichments. For instance, [U-$^{13}C_6$]citrate is the product of [U-$^{13}C_4$]OAA and [1,2-$^{13}C_2$]acetyl-CoA. Supplemental Table S4 compares the predicted steady-state enrichments of Cit_f, Cit_h, Cit_i and Cit_j to those measured in Deconvolution of Citrate Isotopomers. The use of OAA and acetyl-CoA isotopic data overestimates the citrate isotopomer enrichments by ~13%. This consistent overestimation of the enrichments further emphasizes the argument in favor of the equilibration of citrate with αKG through reversed ICDH.

S4 Isotopic Steady-State Relationships

One or more metabolic pathways may contribute substrate to an enzymatic reaction. For instance Acetyl-CoA formation arises primarily from either pyruvate decarboxylation (PDH) or β-oxidation of fatty acids or some amino acids. The relative source of the carbons flowing into an enzymatic pathway can be determined if one of the pathways (e.g. pyruvate dehydrogenation or pyruvate carboxylation) can be selectively labeled. Differential equations can be defined to describe the rate of change of enrichment of a labeled metabolite of a substrate arising from a metabolic inflow minus the disappearance of the product. For the generalized reaction

$$A \overset{E_1}{\rightarrow} B \overset{E_2}{\rightarrow} C$$

where A is the initial substrate converted to product B by enzyme E_1 and then B is converted to C by reaction E_2 then the general equation is

$$\frac{d[FE_{B^*}]}{dt} = FE_{A^*} * \nu_{E_1} - FE_{B^*} * \nu_{E_2} \quad \text{(Eq 37)}$$

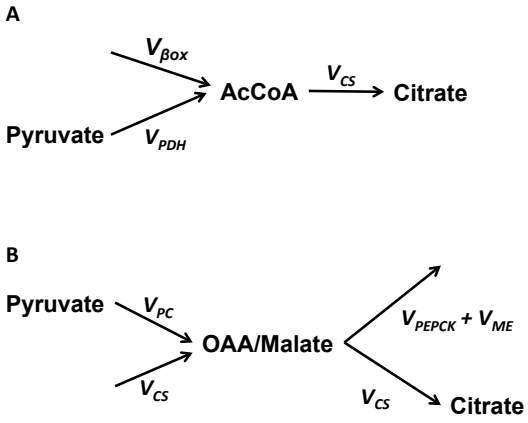
At metabolic and isotopic steady-state, the variation in ^{13}C-enrichment with time is zero by definition. Therefore the relative flux of E_1 with respect to E_2 ($\Phi_{1\rightarrow 2}$) can be solved such that the relative contribution of the input relative to the output is equal to the enrichment of the product to its precursor (Eq 38).

$$\Phi_{1\rightarrow 2} = \frac{\nu_{E_1}}{\nu_{E_2}} = \frac{FE_{B^*}}{FE_{A^*}} \quad \text{(Eq 38)}$$

If ν_{E_1} is the only flux contributing to the generation of B, then no other pathway

contributes to FE_{B^*} and $\frac{v_{E_1}}{v_{E_2}}$ will approach 1. As long as there are no additional metabolic inputs into the product B, then E_1 could describe multiple different reactions along the same pathway. Unfortunately, such a steady-state isotopic analysis can only determine whether or not there are significant net entry or exchange (balanced entry and exit) of an unlabeled metabolite between sequential or tandem metabolic reactions. Values less than 1 indicate unlabeled inputs from another source. For simplicity, we refer to the relative contribution of a substrate from a pathway A to its product pathway B as Φ_{AB} and 1-Φ_{AB}equals the unlabeled input(s) to that pathway as shown below. It is worth noting that this analysis cannot distinguish anaplerotic from exchange reactions and is unable to identify cataplerotic loss of carbon as well.

PDH and PC reactions are the two main entry points of ^{13}C-label into the TCA cycle. At steady state, PDH and PC fluxes can be described relative to CS. Both of these relationships are shown:



The pools of OAA and malate were considered as one single pool due to the high exchange rate between OAA and malate relative to CS (Assumption 5, Methods section).

PDH converts [U-$^{13}C_3$]pyruvate into [1,2-$^{13}C_2$]acetyl-CoA which can, in turn, undergo further oxidation via the TCA cycle reactions (Supplemental Figure S4-A). The variation of [1,2-$^{13}C_2$]acetyl-CoA with time as well as the mass balance relation are described by equations 39

and 40. (As discussed, [U-$^{13}C_3$]PEP better represents the true enrichment of the glycolytic precursors. Therefore, we replaced [U-$^{13}C_3$]pyruvate with [U-$^{13}C_3$]PEP enrichments in all equations that follow (including equation 39).

$$\frac{d[1,2-{}^{13}C_2]acetyl-CoA}{dt} = [U-{}^{13}C_3]PEP * V_{PDH} - [1,2-{}^{13}C_2]AcCoA * V_{CS} \quad \text{(Eq 39)}$$

$$V_{PDH} + V_{\beta ox} = V_{CS} \quad \text{(Eq 40)}$$

At steady state, equation 39 can be further simplified and solved for V_{PDH}/V_{CS} to get the relative flux of PDH to CS (Φ_{PAc})(Eq 41). The enrichment in [1,2-$^{13}C_2$]AcCoA can be calculated as described in "Calculation of Acetyl-CoA Enrichments" while [U-$^{13}C_3$]PEP can be directly measured. Additionally, unlabeled input(s) coming from β-oxidation (or other unlabeled pathways) into mitochondrial acetyl-CoA ($V_{\beta ox}$, Supplemental Figure S4-A) can also be calculated by taking equation 40 into account (Eq. 42).

$$\Phi_{PAc} = \frac{V_{PDH}}{V_{CS}} = \frac{[1,2-{}^{13}C_2]AcCoA}{[U-{}^{13}C_3]PEP} \quad \text{(Eq 41)}$$

$$\Phi_{\beta Ac} = \frac{V_{\beta ox}}{V_{CS}} = 1 - \Phi_{PAc} \quad \text{(Eq 42)}$$

PC is a mitochondrial enzyme that converts pyruvate into OAA (Supplemental Figure S4-B). The relative rate of [(1,2,3)(2,3,4)-$^{13}C_3$]OAA synthesized from [U-$^{13}C_3$]pyruvate can be determined similarly to the described for PDH. In this case, OAA and malate are considered as one pool due to the rapid exchange relative to the TCA cycle flux (Assumption 5, Methods section). It follows that both PEPCK and ME must be accounted for as reactions that consume [(1,2,3)(2,3,4)-$^{13}C_3$]OAA. It should also be noted that it is impossible to distinguish between

OAA coming directly through PC flux from that coming indirectly via reverse malic enzyme flux (pyruvate → malate). Both the variation of [(1,2,3)(2,3,4)-$^{13}C_3$]OAA with time as well as the mass balance relation is described by equations 43 and 44, respectively.

$$\frac{d[(1,2,3)(2,3,4)-{}^{13}C_3]OAA}{dt} = [U-{}^{13}C_3]PEP * V_{PC} - \left[(1,2,3)(2,3,4)-{}^{13}C_3\right]OAA * (V_{CS}+V_{ME}+V_{PEPCK}) \quad \text{(Eq 43)}$$

$$V_{CS}+V_{PC} = V_{CS}+V_{PEPCK}+V_{ME} \quad \Rightarrow \quad V_{PC} = V_{PEPCK}+V_{ME} \quad \text{(Eq 44)}$$

Equation 43 can be re-arranged when we take equation 44 into account (Eq. 45). At steady state, equation 45 can be further simplified and solved for V_{PC}/V_{CS} to get the relative flux of PC to CS (Φ_{PO}) (Eq 46).

$$\frac{d[(1,2,3)(2,3,4)-{}^{13}C_3]OAA}{dt} = [U-{}^{13}C_3]PEP * V_{PC} - \left[(1,2,3)(2,3,4)-{}^{13}C_3\right]OAA * V_{PC} - \left[(1,2,3)(2,3,4)-{}^{13}C_3\right]OAA * V_{CS} \quad \text{(Eq 45)}$$

$$\Phi_{PO} = \frac{V_{PC}}{V_{CS}} = \frac{\left[(1,2,3)(2,3,4)-{}^{13}C_3\right]OAA}{([U-{}^{13}C_3]P\square P-\left[(1,2,3)(2,3,4)-{}^{13}C_3\right]OAA)} \quad \text{(Eq 46)}$$

In addition, V_{PC}/V_{CS} can also be calculated using malate (Φ_{POM}) and aspartate (Φ_{POD}) as surrogate for the enrichments of OAA (Deconvolution of Citrate Isotopomers) (Eq 47-48).

$$\Phi_{POM} = \frac{\left[(1,2,3)(2,3,4)-{}^{13}C_3\right]malate}{([U-{}^{13}C_3]PEP-\left[(1,2,3)(2,3,4)-{}^{13}C_3\right]malate)} \quad \text{(Eq 47)}$$

$$\Phi_{POD} = \frac{\left[(1,2,3)(2,3,4)-{}^{13}C_3\right]aspartate}{([U-{}^{13}C_3]PEP-\left[(1,2,3)(2,3,4)-{}^{13}C_3\right]aspartate)} \quad \text{(Eq 48)}$$

PC and PDH fluxes share the same precursor (pyruvate) that react in the mitochondria

to form a common product (citrate). The relative glycolytic contribution to the TCA cycle of PC vs. PDH (Φ_{PO}/Φ_{PAc}) can be determined from analysis of citrate isotopomers without the enrichment of pyruvate (the input function) since they are the same for both. Dividing the equations simplifies to eliminate the pyruvate enrichment (Eq 49).

$$\frac{\Phi_{PO}}{\Phi_{PAc}} = \frac{[(1,2,3)(2,3,4)-{}^{13}C_3]OAA}{[1,2-{}^{13}C_2]acetyl-CoA} \approx \frac{[(1,2,3)(2,3,4)-{}^{13}C_3]malate}{[1,2-{}^{13}C_2]acetyl-CoA} \approx \frac{[(1,2,3)(2,3,4)-{}^{13}C_3]aspartate}{[1,2-{}^{13}C_2]acetyl-CoA} \quad \text{(Eq 49)}$$

As shown above, the citrate isotopomer family analysis measures the individual populations of isotopomers that contribute to both PC-derived OAA (families $cit_{c,h}$) and PDH-derived Acetyl-CoA (families $cit_{a,d,f,h,i,j}$). Thus the relative PC/PDH flux can be determined by deconvolving the intramolecular isotopomeric labeling of citrate alone (Eq 50). These and other relationships are described in Supplemental Table S2.

$$\Phi_{PO}/\Phi_{PAc} = \frac{cit(c,h)}{cit(a,d,f,i,h,j)} \quad \text{(Eq 50)}$$

A graphical representation of the results from Supplemental Table S2 is shown in Figure S2. Some of the relationships described in Supplemental Table S2 are equivalent, i.e., the ratio between precursor and product should be similar for different isotopomer groups if undergoing the same reaction. This is indicated by the numbered index following the flux description. For example, $\Phi_{CitG1-7}$, describe seven similar ways to characterize the flux from citrate to glutamate. However, because some of these isotopomer groups have low enrichment, the analysis is naturally prone to a certain degree of variability. For this reason, the relationship involving the highest enrichments, from within equivalent relationships, was marked with (*) in Supplemental Figure S2 and used as a reference.

S5. Dynamic Modeling

Flux through citrate synthase (V_{CS}) was determined by fitting a one compartment metabolic model to the labeling time course of the TCA cycle intermediates (Supplemental Figure S4-A). At metabolic steady state, anaplerosis is balanced by cataplerosis. Similarly, molecular exchange in equals exchange out. For the purpose of these measurements: **1)** TCA flux must flow through CS, **2)** PC flux must be balanced (V_{PC}= $V_{PEP/Pyr_{Cycling}}$) and **3)** the entry of glutamate into the TCA cycle by transaminase exchange does not contribute to net TCA flux. The model depicted in Supplemental Figure S4-A was fitted to our ^{13}C-label time courses using the CWave software (Mason et al., 2003).

The model used to calculate V_{CS} is driven by the time course of [U-$^{13}C_3$]PEP and it starts with the conversion of PEP into pyruvate (Supplemental Figure S4-A). In this model, pyruvate enters the TCA cycle via either the pyruvate dehydrogenase (PDH) or pyruvate carboxylase (PC) reactions. The pool of pyruvate is balanced by a cycling reaction, $V_{PEP/Pyr_{Cycling}}$, which converts OAA into pyruvate. Acetyl-CoA is synthesized directly by PDH. The dilution of the acetyl-CoA by oxidation of unlabeled lipid sources is described by $V_{\beta ox}$. $V_{\beta ox}$ was set as (1-Φ_{PAc})* V_{CS} (Supplemental Table S2). Citrate is converted to αKG at a flux equal to V_{CS}. An additional exchange rate is defined between the αKG and the citrate (V_{ICDH}) pools to explain the label dilution between acetyl-CoA and citrate at steady state (Supplemental Figure S2). Several factors were taken into account when choosing the addition of an exchange between citrate and αKG, as opposed to an exchange between citrate and an unlabeled source of carbons. While the only realistic source of dilution of the citrate pool is cytosolic citrate, at steady state, both pools, cytosolic and mitochondrial, are expected to have similar enrichments. Additionally, the quality of the fits of the ^{13}C-citrate time course data is much improved with the addition V_{ICDH}. For instance, the analysis of the time course for labeled

citrate originated from PDH (Σ $Cit_{a,f,i,d,h,j}$) at 9mM glucose shows that it is better fit by multiexponential function (Supplemental Figures S4-C,D). In fact, modeling these data assuming an exchange between citrate and an unlabeled source originates a single exponential function that does not pass through all the data points (Supplemental Figure S4-E). However, when V_{ICDH} is included we obtain a more accurate description of the time course (Supplemental Figure S9-F). Finally, the addition of V_{ICDH} is in agreement with the physiology of the INS-1 cells where the possibility for reversed flux through isocitrate dehydrogenase (ICDH) has been considered (MacDonald et al., 2013).

At the level of αKG, $V_{Glut_{Exc}}$ describes the transaminase reaction between αKG and glutamate. In our model, $V_{Glut_{Exc}}$ was allowed to vary in order to settle in a value that corresponded to a complete exchange between the two pools. However, an upper limit was defined at 100*V_{CS}. The limit of 100*V_{CS} simultaneously allows the model to find a rate that is not limiting relative to CS and reduces the amount of noise that comes from the search of a value of $V_{Glut_{Exc}}$ several magnitudes higher than V_{CS}. The description of the glutamate enrichment data required the addition of a dilution rate. $V_{Glut_{Dil}}$ describes the dilution observed in the glutamate pool relative to the enrichments in citrate (Supplemental Figure S2). This dilution can be explained by the presence of unlabeled glutamine in the experimental media. When unlabeled glutamine is absent from the experimental media the enrichments of citrate approach those of glutamate (Supplemental Figures S3-A,B). Without $V_{Glut_{Dil}}$ the data cannot be fit (data not shown). Finally, V_{SC} refers to the exchange between the OAA and malate/fumarate pools. The goal of this rate is to achieve the racemization of label expected from the equilibrium with fumarate. Therefore, V_{SC} was allowed to vary with an upper limit of 100*V_{CS} similar to what was described for $V_{Glut_{Exc}}$.

The mass balance equations used to describe the variation of the concentration of the

metabolites depicted in Supplemental Figure S4-A are as follows:

$$\frac{d[Pyruvate]}{dt} = V_{PEP/Pyr_{Cycling}} + V_{PK} - (V_{PC} + V_{PDH})$$

$$\frac{d[AcetylCoA]}{dt} = V_{PDH} + V_{\beta ox} - V_{CS}$$

$$\frac{d[Citrate]}{dt} = V_{CS} + V_{ICDH} - (V_{CS} + V_{ICDH})$$

$$\frac{d[\alpha KG]}{dt} = V_{CS} + V_{Glut_{Exc}} + V_{ICDH} - \left(V_{CS} + V_{Glut_{Exc}} + V_{ICDH}\right)$$

$$\frac{d[Glutamate]}{dt} = V_{Glut_{Dil}} + V_{Glut_{Exc}} - (V_{Glut_{Dil}} + V_{Glut_{Exc}})$$

$$\frac{d[Succinate]}{dt} = V_{CS} - V_{CS}$$

$$\frac{d[Malate]}{dt} = V_{CS} + V_{SC} - (V_{CS} + V_{SC})$$

$$\frac{d[OAA]}{dt} = V_{CS} + V_{SC} + V_{PC} - (V_{CS} + V_{SC} + V_{PEP/Pyr_{Cycling}})$$

$$V_{PK} = V_{PDH}$$

$$V_{PEP/Pyr_{Cycling}} = V_{PC}$$

$$V_{CS} = V_{PDH} + V_{\beta ox} = V_{TCA}$$

$$V_{\beta ox} = (1 - \Phi_{PAc}) * V_{CS}$$

A brief description of the label flow within the TCA cycle is shown in Supplemental Figure S4-B. Essentially, all the carbon positions are maintained during the mass transfer from citrate to glutamate. The only exception is carbon 6 of citrate, which is lost during the first decarboxylation occurring in the TCA cycle. Thus, [4,5-$^{13}C_2$]citrate, for instance, originates [4,5-$^{13}C_2$]glutamate (Supplemental Figure S4-B). In the transition from glutamate to succinate, the molecular symmetry of succinate must be taken into account. Because of this symmetry,

[4,5-$^{13}C_2$]glutamate originates equal amounts of [1,2-$^{13}C_2$] and [3,4-$^{13}C_2$]succinate, each formed at rate that is ½ of the TCA cycle flux. A similar concept was used to described the racemization of the label between the OAA, malate and fumarate pools. This racemization occurs because of the near-equilibrium reactions connecting the OAA and malate pools with fumarate, also a symmetrical molecule. Fumarate was not included in this model. Instead, we assumed that racemization occurred in the malate pool. Thus, [1,2-$^{13}C_2$]malate originates [1,2-$^{13}C_2$] and [3,4-$^{13}C_2$]OAA at a rate that is ½ of (V_{CS}+V_{SC}). The isotopic balance equations used to fully describe the ^{13}C-label flow between all possible isotopomers of the considered metabolites are as follows:

Pyruvate

dPyruvate_123/dt = PK(PEP_123/PEP) + PEP/Pyr_cycling(OAA_123/OAA) + PEP/Pyr_cycling(OAA_1234/OAA) - (PDH + PC)(Pyruvate_123/Pyruvate)

dPyruvate_12/dt = PEP/Pyr_cycling(OAA_124/OAA) + PEP/Pyr_cycling(OAA_12/OAA) + PDH(NA_0/NA) - (PDH + PC)(Pyruvate_12/Pyruvate)

dPyruvate_23/dt = PEP/Pyr_cycling(OAA_23/OAA) + PEP/Pyr_cycling(OAA_234/OAA) + PDH(NA_0/NA) - (PDH + PC)(Pyruvate_23/Pyruvate)

dPyruvate_13/dt = PEP/Pyr_cycling(OAA_13/OAA) + PEP/Pyr_cycling(OAA_134/OAA) + PDH(NA_0/NA) - (PDH + PC)(Pyruvate_13/Pyruvate)

dPyruvate_1/dt = PEP/Pyr_cycling(OAA_1/OAA) + PEP/Pyr_cycling(OAA_4/OAA) + PDH(NA_0/NA) - (PDH + PC)(Pyruvate_1/Pyruvate)

dPyruvate_2/dt = PEP/Pyr_cycling(OAA_2/OAA) + PEP/Pyr_cycling(OAA_24/OAA) + PDH(NA_0/NA) - (PDH + PC)(Pyruvate_2/Pyruvate)

dPyruvate_3/dt = PEP/Pyr_cycling(OAA_3/OAA) + PEP/Pyr_cycling(OAA_34/OAA) + PDH(NA_0/NA) - (PDH + PC)(Pyruvate_3/Pyruvate)

Acetyl-CoA

dAcetylCoA_12/dt = PDH(Pyruvate_123/Pyruvate) + PDH(Pyruvate_23/Pyruvate) + Beta_ox(NA_0/NA) - CS(AcetylCoA_12/AcetylCoA)

dAcetylCoA_1/dt = PDH(Pyruvate_12/Pyruvate) + PDH(Pyruvate_2/Pyruvate) + Beta_ox(NA_0/NA) - CS(AcetylCoA_1/AcetylCoA)

dAcetylCoA_2/dt = PDH(Pyruvate_13/Pyruvate) + PDH(Pyruvate_3/Pyruvate) + Beta_ox(NA_0/NA) - CS(AcetylCoA_2/AcetylCoA)

Citrate

dCitrate_1/dt = CS(AcetylCoA_0/AcetylCoA)(OAA_1/OAA) + ICDH(aKG_1/aKG) - (CS + ICDH)(Citrate_1/Citrate)

dCitrate_2/dt = CS(OAA_2/OAA)(AcetylCoA_0/AcetylCoA) + ICDH(aKG_2/aKG) - (CS + ICDH)(Citrate_2/Citrate)

dCitrate_3/dt = CS(OAA_3/OAA)(AcetylCoA_0/AcetylCoA) + ICDH(aKG_3/aKG) - (CS + ICDH)(Citrate_3/Citrate)

dCitrate_4/dt = CS(AcetylCoA_2/AcetylCoA)(OAA_0/OAA) + ICDH(aKG_4/aKG) - (CS + ICDH)(Citrate_4/Citrate)

dCitrate_5/dt = CS(AcetylCoA_1/AcetylCoA)(OAA_0/OAA) + ICDH(aKG_5/aKG) - (CS + ICDH)(Citrate_5/Citrate)

dCitrate_6/dt = CS(OAA_4/OAA)(OAA_0/OAA) + ICDH(NA_0/NA) - (CS + ICDH)(Citrate_6/Citrate)

dCitrate_12/dt = CS(OAA_12/OAA)(OAA_0/OAA) + ICDH(aKG_12/aKG) - (CS + ICDH)(Citrate_12/Citrate)

dCitrate_13/dt = CS(OAA_13/OAA)(AcetylCoA_0/AcetylCoA) + ICDH(aKG_13/aKG) - (CS + ICDH)(Citrate_13/Citrate)

dCitrate_14/dt = CS(AcetylCoA_2/AcetylCoA)(OAA_1/OAA) + ICDH(aKG_14/aKG) - (CS + ICDH)(Citrate_14/Citrate)

dCitrate_15/dt = CS(AcetylCoA_1/AcetylCoA)(OAA_1/OAA) + ICDH(aKG_15/aKG) - (CS + ICDH)(Citrate_15/Citrate)

dCitrate_16/dt = CS(OAA_14/OAA)(AcetylCoA_0/AcetylCoA) + ICDH(NA_0/NA) - (CS + ICDH)(Citrate_16/Citrate)

dCitrate_23/dt = CS(OAA_23/OAA)(AcetylCoA_0/AcetylCoA) + ICDH(aKG_23/aKG) - (CS + ICDH)(Citrate_23/Citrate)

dCitrate_24/dt = CS(OAA_2/OAA)(AcetylCoA_2/AcetylCoA) + ICDH(aKG_24/aKG) - (CS + ICDH)(Citrate_24/Citrate)

dCitrate_25/dt = CS(OAA_2/OAA)(AcetylCoA_1/AcetylCoA) + ICDH(aKG_25/aKG) - (CS + ICDH)(Citrate_25/Citrate)

dCitrate_26/dt = CS(OAA_24/OAA)(AcetylCoA_0/AcetylCoA) + ICDH(NA_0/NA) - (CS + ICDH)(Citrate_26/Citrate)

dCitrate_34/dt = CS(OAA_3/OAA)(AcetylCoA_2/AcetylCoA) + ICDH(aKG_34/aKG) - (CS + ICDH)(Citrate_34/Citrate)

dCitrate_35/dt = CS(OAA_3/OAA)(AcetylCoA_1/AcetylCoA) + ICDH(aKG_35/aKG) - (CS + ICDH)(Citrate_35/Citrate)

dCitrate_36/dt = CS(OAA_34/OAA)(AcetylCoA_0/AcetylCoA) + ICDH(NA_0/NA) - (CS + ICDH)(Citrate_36/Citrate)

dCitrate_45/dt = CS(AcetylCoA_12/AcetylCoA)(OAA_0/OAA) + ICDH(aKG_45/aKG) - (CS + ICDH)(Citrate_45/Citrate)

dCitrate_46/dt = CS(AcetylCoA_2/AcetylCoA)(OAA_4/OAA) + ICDH(NA_0/NA) - (CS + ICDH)(Citrate_46/Citrate)

dCitrate_56/dt = CS(AcetylCoA_1/AcetylCoA)(OAA_4/OAA) + ICDH(NA_0/NA) - (CS + ICDH)(Citrate_56/Citrate)

dCitrate_123/dt = CS(OAA_123/OAA)(AcetylCoA_0/AcetylCoA) + ICDH(aKG_123/aKG) - (CS + ICDH)(Citrate_123/Citrate)

dCitrate_124/dt = CS(AcetylCoA_2/AcetylCoA)(OAA_12/OAA) + ICDH(aKG_124/aKG) - (CS + ICDH)(Citrate_124/Citrate)

dCitrate_125/dt = CS(AcetylCoA_1/AcetylCoA)(OAA_12/OAA) + ICDH(aKG_125/aKG) - (CS + ICDH)(Citrate_125/Citrate)

dCitrate_126/dt = CS(OAA_124/OAA)(AcetylCoA_0/AcetylCoA) + ICDH(NA_0/NA) - (CS + ICDH)(Citrate_126/Citrate)

dCitrate_134/dt = CS(AcetylCoA_2/AcetylCoA)(OAA_13/OAA) + ICDH(aKG_134/aKG) - (CS + ICDH)(Citrate_134/Citrate)

dCitrate_135/dt = CS(AcetylCoA_1/AcetylCoA)(OAA_13/OAA) + ICDH(aKG_135/aKG) - (CS + ICDH)(Citrate_135/Citrate)

dCitrate_136/dt = CS(OAA_134/OAA)(AcetylCoA_0/AcetylCoA) + ICDH(NA_0/NA) - (CS + ICDH)(Citrate_136/Citrate)

dCitrate_145/dt = CS(AcetylCoA_12/AcetylCoA)(OAA_1/OAA) + ICDH(aKG_145/aKG) - (CS + ICDH)(Citrate_145/Citrate)

dCitrate_146/dt = CS(AcetylCoA_2/AcetylCoA)(OAA_14/OAA) + ICDH(NA_0/NA) - (CS + ICDH)(Citrate_146/Citrate)

dCitrate_156/dt = CS(AcetylCoA_1/AcetylCoA)(OAA_14/OAA) + ICDH(NA_0/NA) - (CS + ICDH)(Citrate_156/Citrate)

dCitrate_234/dt = CS(AcetylCoA_2/AcetylCoA)(OAA_23/OAA) + ICDH(aKG_234/aKG) - (CS + ICDH)(Citrate_234/Citrate)

dCitrate_235/dt = CS(AcetylCoA_1/AcetylCoA)(OAA_23/OAA) + ICDH(aKG_235/aKG) - (CS + ICDH)(Citrate_235/Citrate)

dCitrate_236/dt = CS(OAA_234/OAA)(AcetylCoA_0/AcetylCoA) + ICDH(NA_0/NA) - (CS + ICDH)(Citrate_236/Citrate)

dCitrate_245/dt = CS(AcetylCoA_12/AcetylCoA)(OAA_2/OAA) + ICDH(aKG_245/aKG) - (CS + ICDH)(Citrate_245/Citrate)

dCitrate_246/dt = CS(AcetylCoA_2/AcetylCoA)(OAA_24/OAA) + ICDH(NA_0/NA) - (CS + ICDH)(Citrate_246/Citrate)

dCitrate_256/dt = CS(AcetylCoA_1/AcetylCoA)(OAA_24/OAA) + ICDH(NA_0/NA) - (CS + ICDH)(Citrate_256/Citrate)

dCitrate_345/dt = CS(AcetylCoA_12/AcetylCoA)(OAA_3/OAA) + ICDH(aKG_345/aKG) - (CS + ICDH)(Citrate_345/Citrate)

dCitrate_346/dt = CS(AcetylCoA_2/AcetylCoA)(OAA_34/OAA) + ICDH(NA_0/NA) - (CS + ICDH)(Citrate_346/Citrate)

dCitrate_356/dt = CS(AcetylCoA_1/AcetylCoA)(OAA_34/OAA) + ICDH(NA_0/NA) - (CS + ICDH)(Citrate_356/Citrate)

dCitrate_456/dt = CS(AcetylCoA_12/AcetylCoA)(OAA_4/OAA) + ICDH(NA_0/NA) - (CS + ICDH)(Citrate_456/Citrate)

dCitrate_1234/dt = CS(AcetylCoA_2/AcetylCoA)(OAA_123/OAA) + ICDH(aKG_1234/aKG) - (CS + ICDH)(Citrate_1234/Citrate)

dCitrate_1235/dt = CS(AcetylCoA_1/AcetylCoA)(OAA_123/OAA) + ICDH(aKG_1235/aKG) - (CS + ICDH)(Citrate_1235/Citrate)

dCitrate_1236/dt = CS(OAA_1234/OAA)(AcetylCoA_0/AcetylCoA) + ICDH(NA_0/NA) - (CS + ICDH)(Citrate_1236/Citrate)

dCitrate_1245/dt = CS(AcetylCoA_12/AcetylCoA)(OAA_12/OAA) + ICDH(aKG_1245/aKG) - (CS + ICDH)(Citrate_1245/Citrate)

dCitrate_1246/dt = CS(AcetylCoA_2/AcetylCoA)(OAA_124/OAA) + ICDH(NA_0/NA) - (CS + ICDH)(Citrate_1246/Citrate)

dCitrate_1256/dt = CS(AcetylCoA_1/AcetylCoA)(OAA_124/OAA) + ICDH(NA_0/NA) - (CS + ICDH)(Citrate_1256/Citrate)

dCitrate_1345/dt = CS(AcetylCoA_12/AcetylCoA)(OAA_13/OAA) + ICDH(aKG_1345/aKG) - (CS + ICDH)(Citrate_1345/Citrate)

dCitrate_1346/dt = CS(AcetylCoA_2/AcetylCoA)(OAA_134/OAA) + ICDH(NA_0/NA) - (CS + ICDH)(Citrate_1346/Citrate)

dCitrate_1356/dt = CS(AcetylCoA_1/AcetylCoA)(OAA_134/OAA) + ICDH(NA_0/NA) - (CS + ICDH)(Citrate_1356/Citrate)

dCitrate_1456/dt = CS(AcetylCoA_12/AcetylCoA)(OAA_14/OAA) + ICDH(NA_0/NA) - (CS + ICDH)(Citrate_1456/Citrate)

dCitrate_2345/dt = CS(AcetylCoA_12/AcetylCoA)(OAA_23/OAA) + ICDH(aKG_2345/aKG) - (CS + ICDH)(Citrate_2345/Citrate)

dCitrate_2346/dt = CS(AcetylCoA_2/AcetylCoA)(OAA_234/OAA) + ICDH(NA_0/NA) - (CS + ICDH)(Citrate_2346/Citrate)

dCitrate_2356/dt = CS(AcetylCoA_1/AcetylCoA)(OAA_234/OAA) + ICDH(NA_0/NA) - (CS + ICDH)(Citrate_2356/Citrate)

dCitrate_2456/dt = CS(AcetylCoA_12/AcetylCoA)(OAA_24/OAA) + ICDH(NA_0/NA) - (CS + ICDH)(Citrate_2456/Citrate)

dCitrate_3456/dt = CS(AcetylCoA_12/AcetylCoA)(OAA_34/OAA) + ICDH(NA_0/NA) - (CS + ICDH)(Citrate_3456/Citrate)

dCitrate_12345/dt = CS(AcetylCoA_12/AcetylCoA)(OAA_123/OAA) + ICDH(aKG_12345/aKG) - (CS + ICDH)(Citrate_12345/Citrate)

dCitrate_12346/dt = CS(AcetylCoA_2/AcetylCoA)(OAA_1234/OAA) + ICDH(NA_0/NA) - (CS + ICDH)(Citrate_12346/Citrate)

dCitrate_12356/dt = CS(AcetylCoA_1/AcetylCoA)(OAA_1234/OAA) + ICDH(NA_0/NA) - (CS + ICDH)(Citrate_12356/Citrate)

dCitrate_12456/dt = CS(AcetylCoA_12/AcetylCoA)(OAA_124/OAA) + ICDH(NA_0/NA) - (CS + ICDH)(Citrate_12456/Citrate)

dCitrate_13456/dt = CS(AcetylCoA_12/AcetylCoA)(OAA_134/OAA) + ICDH(NA_0/NA) - (CS + ICDH)(Citrate_13456/Citrate)

dCitrate_23456/dt = CS(AcetylCoA_12/AcetylCoA)(OAA_234/OAA) + ICDH(NA_0/NA) - (CS + ICDH)(Citrate_23456/Citrate)

dCitrate_123456/dt = CS(AcetylCoA_12/AcetylCoA)(OAA_1234/OAA) + ICDH(NA_0/NA) - (CS + ICDH)(Citrate_123456/Citrate)

αKG

daKG_1/dt = CS(Citrate_1/Citrate) + CS(Citrate_16/Citrate) + Glut_exc(Glutamate_1/Glutamate) + ICDH(Citrate_1/Citrate) + ICDH(Citrate_16/Citrate) - (CS + Glut_exc + ICDH)(aKG_1/aKG)

daKG_2/dt = CS(Citrate_2/Citrate) + CS(Citrate_26/Citrate) + Glut_exc(Glutamate_2/Glutamate) + ICDH(Citrate_2/Citrate) + ICDH(Citrate_26/Citrate) - (CS + Glut_exc + ICDH)(aKG_2/aKG)

daKG_3/dt = CS(Citrate_3/Citrate) + CS(Citrate_36/Citrate) + Glut_exc(Glutamate_2/Glutamate) + ICDH(Citrate_3/Citrate) + ICDH(Citrate_36/Citrate) - (CS + Glut_exc + ICDH)(aKG_3/aKG)

daKG_4/dt = CS(Citrate_4/Citrate) + CS(Citrate_46/Citrate) + Glut_exc(Glutamate_4/Glutamate) + ICDH(Citrate_4/Citrate) + ICDH(Citrate_46/Citrate) - (CS + Glut_exc + ICDH)(aKG_4/aKG)

daKG_5/dt = CS(Citrate_5/Citrate) + CS(Citrate_56/Citrate) + Glut_exc(Glutamate_5/Glutamate) + ICDH(Citrate_5/Citrate) + ICDH(Citrate_56/Citrate) - (CS + Glut_exc + ICDH)(aKG_5/aKG)

daKG_12/dt = CS(Citrate_12/Citrate) + CS(Citrate_126/Citrate) + Glut_exc(Glutamate_12/Glutamate) + ICDH(Citrate_126/Citrate) + ICDH(Citrate_12/Citrate) - (CS + Glut_exc + ICDH)(aKG_12/aKG)

daKG_13/dt = CS(Citrate_13/Citrate) + CS(Citrate_136/Citrate) + Glut_exc(Glutamate_13/Glutamate) + ICDH(Citrate_13/Citrate) + ICDH(Citrate_136/Citrate) - (CS + Glut_exc + ICDH)(aKG_13/aKG)

daKG_14/dt = CS(Citrate_14/Citrate) + CS(Citrate_146/Citrate) + Glut_exc(Glutamate_14/Glutamate) + ICDH(Citrate_14/Citrate) + ICDH(Citrate_146/Citrate) - (CS + Glut_exc + ICDH)(aKG_14/aKG)

daKG_15/dt = CS(Citrate_15/Citrate) + CS(Citrate_156/Citrate) + Glut_exc(Glutamate_15/Glutamate) + ICDH(Citrate_15/Citrate) + ICDH(Citrate_156/Citrate) - (CS + Glut_exc + ICDH)(aKG_15/aKG)

daKG_23/dt = CS(Citrate_23/Citrate) + CS(Citrate_236/Citrate) + Glut_exc(Glutamate_23/Glutamate) + ICDH(Citrate_23/Citrate) + ICDH(Citrate_236/Citrate) - (CS + Glut_exc + ICDH)(aKG_23/aKG)

daKG_24/dt = CS(Citrate_24/Citrate) + CS(Citrate_246/Citrate) + Glut_exc(Glutamate_24/Glutamate) + ICDH(Citrate_24/Citrate) + ICDH(Citrate_246/Citrate) - (CS + Glut_exc + ICDH)(aKG_24/aKG)

daKG_25/dt = CS(Citrate_25/Citrate) + CS(Citrate_256/Citrate) + Glut_exc(Glutamate_25/Glutamate) + ICDH(Citrate_25/Citrate) + ICDH(Citrate_256/Citrate) - (CS + Glut_exc + ICDH)(aKG_25/aKG)

daKG_34/dt = CS(Citrate_34/Citrate) + CS(Citrate_346/Citrate) + Glut_exc(Glutamate_34/Glutamate) + ICDH(Citrate_34/Citrate) + ICDH(Citrate_346/Citrate) - (CS + Glut_exc + ICDH)(aKG_34/aKG)

daKG_35/dt = CS(Citrate_35/Citrate) + CS(Citrate_356/Citrate) + Glut_exc(Glutamate_35/Glutamate) + ICDH(Citrate_35/Citrate) + ICDH(Citrate_356/Citrate) - (CS + Glut_exc + ICDH)(aKG_35/aKG)

daKG_45/dt = CS(Citrate_45/Citrate) + CS(Citrate_456/Citrate) + Glut_exc(Glutamate_45/Glutamate) + ICDH(Citrate_45/Citrate) + ICDH(Citrate_456/Citrate) - (CS + Glut_exc + ICDH)(aKG_45/aKG)

daKG_123/dt = CS(Citrate_123/Citrate) + CS(Citrate_1236/Citrate) + Glut_exc(Glutamate_123/Glutamate) + ICDH(Citrate_123/Citrate) + ICDH(Citrate_1236/Citrate) - (CS + Glut_exc + ICDH)(aKG_123/aKG)

daKG_124/dt = CS(Citrate_124/Citrate) + CS(Citrate_1246/Citrate) + Glut_exc(Glutamate_124/Glutamate) + ICDH(Citrate_124/Citrate) + ICDH(Citrate_1246/Citrate) - (CS + Glut_exc + ICDH)(aKG_124/aKG)

daKG_125/dt = CS(Citrate_125/Citrate) + CS(Citrate_1256/Citrate) + Glut_exc(Glutamate_125/Glutamate) + ICDH(Citrate_125/Citrate) + ICDH(Citrate_1256/Citrate) - (CS + Glut_exc + ICDH)(aKG_125/aKG)

daKG_134/dt = CS(Citrate_134/Citrate) + CS(Citrate_1346/Citrate) + Glut_exc(Glutamate_134/Glutamate) + ICDH(Citrate_134/Citrate) + ICDH(Citrate_1346/Citrate) - (CS + Glut_exc + ICDH)(aKG_134/aKG)

daKG_135/dt = CS(Citrate_135/Citrate) + CS(Citrate_1356/Citrate) + Glut_exc(Glutamate_135/Glutamate) + ICDH(Citrate_135/Citrate) + ICDH(Citrate_1356/Citrate) - (CS + Glut_exc + ICDH)(aKG_135/aKG)

daKG_145/dt = CS(Citrate_145/Citrate) + CS(Citrate_1456/Citrate) + Glut_exc(Glutamate_145/Glutamate) + ICDH(Citrate_145/Citrate) + ICDH(Citrate_1456/Citrate) - (CS + Glut_exc + ICDH)(aKG_145/aKG)

daKG_234/dt = CS(Citrate_234/Citrate) + CS(Citrate_2346/Citrate) + Glut_exc(Glutamate_234/Glutamate) + ICDH(Citrate_234/Citrate) + ICDH(Citrate_2346/Citrate) - (CS + Glut_exc + ICDH)(aKG_234/aKG)

daKG_235/dt = CS(Citrate_235/Citrate) + CS(Citrate_2356/Citrate) + Glut_exc(Glutamate_235/Glutamate) + ICDH(Citrate_235/Citrate) + ICDH(Citrate_2356/Citrate) - (CS + Glut_exc + ICDH)(aKG_235/aKG)

daKG_245/dt = CS(Citrate_245/Citrate) + CS(Citrate_2456/Citrate) + Glut_exc(Glutamate_245/Glutamate) + ICDH(Citrate_245/Citrate) + ICDH(Citrate_2456/Citrate) - (CS + Glut_exc + ICDH)(aKG_245/aKG)

daKG_345/dt = CS(Citrate_345/Citrate) + CS(Citrate_3456/Citrate) + Glut_exc(Glutamate_345/Glutamate) + ICDH(Citrate_345/Citrate) + ICDH(Citrate_3456/Citrate) - (CS + Glut_exc + ICDH)(aKG_345/aKG)

daKG_1234/dt = CS(Citrate_1234/Citrate) + CS(Citrate_12346/Citrate) + Glut_exc(Glutamate_1234/Glutamate) + ICDH(Citrate_1234/Citrate) + ICDH(Citrate_12346/Citrate) - (CS + Glut_exc + ICDH)(aKG_1234/aKG)

daKG_1235/dt = CS(Citrate_1235/Citrate) + CS(Citrate_12356/Citrate) + Glut_exc(Glutamate_1235/Glutamate) + ICDH(Citrate_1235/Citrate) + ICDH(Citrate_12356/Citrate) - (CS + Glut_exc + ICDH)(aKG_1235/aKG)

daKG_1245/dt = CS(Citrate_1245/Citrate) + CS(Citrate_12456/Citrate) + Glut_exc(Glutamate_1245/Glutamate) + ICDH(Citrate_1245/Citrate) + ICDH(Citrate_12456/Citrate) - (CS + Glut_exc + ICDH)(aKG_1245/aKG)

daKG_1345/dt = CS(Citrate_1345/Citrate) + CS(Citrate_13456/Citrate) + Glut_exc(Glutamate_1345/Glutamate) + ICDH(Citrate_1345/Citrate) + ICDH(Citrate_13456/Citrate) - (CS + Glut_exc + ICDH)(aKG_1345/aKG)

daKG_2345/dt = CS(Citrate_2345/Citrate) + CS(Citrate_23456/Citrate) + Glut_exc(Glutamate_2345/Glutamate) + ICDH(Citrate_2345/Citrate) + ICDH(Citrate_23456/Citrate) - (CS + Glut_exc + ICDH)(aKG_2345/aKG)

daKG_12345/dt = CS(Citrate_12345/Citrate) + CS(Citrate_123456/Citrate) + Glut_exc(Glutamate_12345/Glutamate) + ICDH(Citrate_12345/Citrate) + ICDH(Citrate_123456/Citrate) - (CS + Glut_exc + ICDH)(aKG_12345/aKG)

Glutamate

dGlutamate_1/dt = Glut_dil(NA_0/NA) + Glut_exc(aKG_1/aKG) - (Glut_dil + Glut_exc)(Glutamate_1/Glutamate)

dGlutamate_2/dt = Glut_dil(NA_0/NA) + Glut_exc(aKG_2/aKG) - (Glut_dil + Glut_exc)(Glutamate_2/Glutamate)

dGlutamate_3/dt = Glut_dil(NA_0/NA) + Glut_exc(aKG_3/aKG) - (Glut_dil + Glut_exc)(Glutamate_3/Glutamate)

dGlutamate_4/dt = Glut_dil(NA_0/NA) + Glut_exc(aKG_4/aKG) - (Glut_dil + Glut_exc)(Glutamate_4/Glutamate)

dGlutamate_5/dt = Glut_dil(NA_0/NA) + Glut_exc(aKG_5/aKG) - (Glut_dil + Glut_exc)(Glutamate_5/Glutamate)

dGlutamate_12/dt = Glut_dil(NA_0/NA) + Glut_exc(aKG_12/aKG) - (Glut_dil + Glut_exc)(Glutamate_12/Glutamate)

dGlutamate_13/dt = Glut_dil(NA_0/NA) + Glut_exc(aKG_13/aKG) - (Glut_dil + Glut_exc)(Glutamate_13/Glutamate)

dGlutamate_14/dt = Glut_dil(NA_0/NA) + Glut_exc(aKG_14/aKG) - (Glut_dil + Glut_exc)(Glutamate_14/Glutamate)

dGlutamate_15/dt = Glut_dil(NA_0/NA) + Glut_exc(aKG_15/aKG) - (Glut_dil + Glut_exc)(Glutamate_15/Glutamate)

dGlutamate_23/dt = Glut_dil(NA_0/NA) + Glut_exc(aKG_23/aKG) - (Glut_dil + Glut_exc)(Glutamate_23/Glutamate)

dGlutamate_24/dt = Glut_dil(NA_0/NA) + Glut_exc(aKG_24/aKG) - (Glut_dil + Glut_exc)(Glutamate_24/Glutamate)

dGlutamate_25/dt = Glut_dil(NA_0/NA) + Glut_exc(aKG_25/aKG) - (Glut_dil + Glut_exc)(Glutamate_25/Glutamate)

dGlutamate_34/dt = Glut_dil(NA_0/NA) + Glut_exc(aKG_34/aKG) - (Glut_dil + Glut_exc)(Glutamate_34/Glutamate)

dGlutamate_35/dt = Glut_dil(NA_0/NA) + Glut_exc(aKG_35/aKG) - (Glut_dil + Glut_exc)(Glutamate_35/Glutamate)

dGlutamate_45/dt = Glut_dil(NA_0/NA) + Glut_exc(aKG_45/aKG) - (Glut_dil + Glut_exc)(Glutamate_45/Glutamate)

dGlutamate_123/dt = Glut_dil(NA_0/NA) + Glut_exc(aKG_123/aKG) - (Glut_dil + Glut_exc)(Glutamate_123/Glutamate)

dGlutamate_124/dt = Glut_dil(NA_0/NA) + Glut_exc(aKG_124/aKG) - (Glut_dil + Glut_exc)(Glutamate_124/Glutamate)

dGlutamate_125/dt = Glut_dil(NA_0/NA) + Glut_exc(aKG_125/aKG) - (Glut_dil + Glut_exc)(Glutamate_125/Glutamate)

dGlutamate_134/dt = Glut_dil(NA_0/NA) + Glut_exc(aKG_134/aKG) - (Glut_dil + Glut_exc)(Glutamate_134/Glutamate)

dGlutamate_135/dt = Glut_dil(NA_0/NA) + Glut_exc(aKG_135/aKG) - (Glut_dil + Glut_exc)(Glutamate_135/Glutamate)

dGlutamate_145/dt = Glut_dil(NA_0/NA) + Glut_exc(aKG_145/aKG) - (Glut_dil + Glut_exc)(Glutamate_145/Glutamate)

dGlutamate_234/dt = Glut_dil(NA_0/NA) + Glut_exc(aKG_234/aKG) - (Glut_dil + Glut_exc)(Glutamate_234/Glutamate)

dGlutamate_235/dt = Glut_dil(NA_0/NA) + Glut_exc(aKG_235/aKG) - (Glut_dil + Glut_exc)(Glutamate_235/Glutamate)

dGlutamate_245/dt = Glut_dil(NA_0/NA) + Glut_exc(aKG_245/aKG) - (Glut_dil + Glut_exc)(Glutamate_245/Glutamate)

dGlutamate_345/dt = Glut_dil(NA_0/NA) + Glut_exc(aKG_345/aKG) - (Glut_dil + Glut_exc)(Glutamate_345/Glutamate)

dGlutamate_1234/dt = Glut_dil(NA_0/NA) + Glut_exc(aKG_1234/aKG) - (Glut_dil + Glut_exc)(Glutamate_1234/Glutamate)

dGlutamate_1235/dt = Glut_dil(NA_0/NA) + Glut_exc(aKG_1235/aKG) - (Glut_dil + Glut_exc)(Glutamate_1235/Glutamate)

dGlutamate_1245/dt = Glut_dil(NA_0/NA) + Glut_exc(aKG_1245/aKG) - (Glut_dil + Glut_exc)(Glutamate_1245/Glutamate)

dGlutamate_1345/dt = Glut_dil(NA_0/NA) + Glut_exc(aKG_1345/aKG) - (Glut_dil + Glut_exc)(Glutamate_1345/Glutamate)

dGlutamate_2345/dt = Glut_dil(NA_0/NA) + Glut_exc(aKG_2345/aKG) - (Glut_dil + Glut_exc)(Glutamate_2345/Glutamate)

dGlutamate_12345/dt = Glut_dil(NA_0/NA) + Glut_exc(aKG_12345/aKG) - (Glut_dil + Glut_exc)(Glutamate_12345/Glutamate)

Succinate

dSuccinate_1/dt = 0.5CS(aKG_12/aKG) + 0.5CS(aKG_15/aKG) + 0.5CS(aKG_5/aKG) + 0.5CS(aKG_2/aKG) - CS(Succinate_1/Succinate)

dSuccinate_2/dt = 0.5CS(aKG_4/aKG) + 0.5CS(aKG_14/aKG) + 0.5CS(aKG_3/aKG) + 0.5CS(aKG_13/aKG) - CS(Succinate_2/Succinate)

dSuccinate_3/dt = 0.5CS(aKG_4/aKG) + 0.5CS(aKG_3/aKG) + 0.5CS(aKG_13/aKG) + 0.5CS(aKG_14/aKG) - CS(Succinate_3/Succinate)

dSuccinate_4/dt = 0.5CS(aKG_5/aKG) + 0.5CS(aKG_2/aKG) + 0.5CS(aKG_12/aKG) + 0.5CS(aKG_15/aKG) - CS(Succinate_4/Succinate)

dSuccinate_12/dt = 0.5CS(aKG_23/aKG) + 0.5CS(aKG_45/aKG) + 0.5CS(aKG_145/aKG) + 0.5CS(aKG_123/aKG) - CS(Succinate_12/Succinate)

dSuccinate_13/dt = 0.5CS(aKG_24/aKG) + 0.5CS(aKG_35/aKG) + 0.5CS(aKG_124/aKG) + 0.5CS(aKG_135/aKG) - CS(Succinate_13/Succinate)

dSuccinate_14/dt = CS(aKG_25/aKG) + CS(aKG_125/aKG) - CS(Succinate_14/Succinate)

dSuccinate_23/dt = CS(aKG_34/aKG) + CS(aKG_134/aKG) - CS(Succinate_23/Succinate)

dSuccinate_24/dt = 0.5CS(aKG_35/aKG) + 0.5CS(aKG_24/aKG) + 0.5CS(aKG_124/aKG) + 0.5CS(aKG_135/aKG) - CS(Succinate_24/Succinate)

dSuccinate_34/dt = 0.5CS(aKG_45/aKG) + 0.5CS(aKG_23/aKG) + 0.5CS(aKG_145/aKG) + 0.5CS(aKG_123/aKG) - CS(Succinate_34/Succinate)

dSuccinate_123/dt = 0.5CS(aKG_234/aKG) + 0.5CS(aKG_345/aKG) + 0.5CS(aKG_1234/aKG) + 0.5CS(aKG_1345/aKG) - CS(Succinate_123/Succinate)

dSuccinate_124/dt = 0.5CS(aKG_235/aKG) + 0.5CS(aKG_245/aKG) + 0.5CS(aKG_1245/aKG) + 0.5CS(aKG_1235/aKG) - CS(Succinate_124/Succinate)

dSuccinate_134/dt = 0.5CS(aKG_235/aKG) + 0.5CS(aKG_245/aKG) + 0.5CS(aKG_1245/aKG) + 0.5CS(aKG_1235/aKG) - CS(Succinate_134/Succinate)

dSuccinate_234/dt = 0.5CS(aKG_234/aKG) + 0.5CS(aKG_345/aKG) + 0.5CS(aKG_1234/aKG) + 0.5CS(aKG_1345/aKG) - CS(Succinate_234/Succinate)

dSuccinate_1234/dt = CS(aKG_12345/aKG) + CS(aKG_2345/aKG) - CS(Succinate_1234/Succinate)

Malate

dMalate_1/dt = 0.5CS(Succinate_1/Succinate) + 0.5CS(Succinate_4/Succinate) + 0.5SC(OAA_1/OAA) + 0.5SC(OAA_4/OAA) - (CS+ SC)(Malate_1/Malate)

dMalate_2/dt = 0.5CS(Succinate_2/Succinate) + 0.5CS(Succinate_3/Succinate) + 0.5SC(OAA_2/OAA) + 0.5SC(OAA_3/OAA) - (CS+SC)(Malate_2/Malate)

dMalate_3/dt = 0.5CS(Succinate_3/Succinate) + 0.5CS(Succinate_2/Succinate) + 0.5SC(OAA_3/OAA) + 0.5SC(OAA_2/OAA) - (CS+SC)(Malate_3/Malate)

dMalate_4/dt = 0.5CS(Succinate_1/Succinate) + 0.5CS(Succinate_4/Succinate) + 0.5SC(OAA_4/OAA) + 0.5SC(OAA_1/OAA) - (CS+SC)(Malate_4/Malate)

dMalate_12/dt = 0.5CS(Succinate_12/Succinate) + 0.5CS(Succinate_34/Succinate) + 0.5SC(OAA_12/OAA) + 0.5SC(OAA_34/OAA) - (CS+SC)(Malate_12/Malate)

dMalate_13/dt = 0.5CS(Succinate_13/Succinate) + 0.5CS(Succinate_24/Succinate) + 0.5SC(OAA_13/OAA) + 0.5SC(OAA_24/OAA) - (CS+SC)(Malate_13/Malate)

dMalate_14/dt = CS(Succinate_14/Succinate) + SC(OAA_14/OAA) - (CS+SC)(Malate_14/Malate)

dMalate_23/dt = CS(Succinate_23/Succinate) + SC(OAA_23/OAA) - (CS+SC)(Malate_23/Malate)

dMalate_24/dt = 0.5CS(Succinate_24/Succinate) + 0.5CS(Succinate_13/Succinate) + 0.5SC(OAA_24/OAA) + 0.5SC(OAA_13/OAA) - (CS+SC)(Malate_24/Malate)

dMalate_34/dt = 0.5CS(Succinate_34/Succinate) + 0.5CS(Succinate_12/Succinate) + 0.5SC(OAA_12/OAA) + 0.5SC(OAA_34/OAA) - (CS+SC)(Malate_34/Malate)

dMalate_123/dt = 0.5CS(Succinate_123/Succinate) + 0.5CS(Succinate_234/Succinate) + 0.5SC(OAA_123/OAA) + 0.5SC(OAA_234/OAA) - (CS+SC)(Malate_123/Malate)

dMalate_234/dt = 0.5CS(Succinate_234/Succinate) + 0.5CS(Succinate_123/Succinate) + 0.5SC(OAA_123/OAA) + 0.5SC(OAA_234/OAA) - (CS+SC)(Malate_234/Malate)

dMalate_124/dt = 0.5CS(Succinate_124/Succinate) + 0.5CS(Succinate_134/Succinate) + 0.5SC(OAA_124/OAA) + 0.5SC(OAA_134/OAA) - (CS+SC)(Malate_124/Malate)

dMalate_134/dt = 0.5CS(Succinate_134/Succinate) + 0.5CS(Succinate_124/Succinate) + 0.5SC(OAA_134/OAA) + 0.5SC(OAA_124/OAA) - (CS+SC)(Malate_134/Malate)

dMalate_1234/dt = CS(Succinate_1234/Succinate) + SC(OAA_1234/OAA) - (CS+SC)(Malate_1234/Malate)

OAA

dOAA_123/dt = PC(Pyruvate_123/Pyruvate) + 0.5(CS+SC)(Malate_123/Malate) + 0.5(CS+SC)(Malate_234/Malate) - (CS + PEP/Pyr_cycling+ SC)(OAA_123/OAA)

dOAA_234/dt = PC(NA_0/NA) + 0.5(CS+SC)(Malate_234/Malate) + 0.5(CS+SC)(Malate_123/Malate) - (CS + PEP/Pyr_cycling+ SC)(OAA_234/OAA)

dOAA_124/dt = PC(NA_0/NA) + 0.5(CS+SC)(Malate_124/Malate) + 0.5(CS+SC)(Malate_134/Malate) - (CS + PEP/Pyr_cycling+ SC)(OAA_124/OAA)

dOAA_134/dt = PC(NA_0/NA) + 0.5(CS+SC)(Malate_134/Malate) + 0.5(CS+SC)(Malate_124/Malate) - (CS + PEP/Pyr_cycling+ SC)(OAA_134/OAA)

dOAA_1234/dt = PC(NA_0/NA) + (CS+SC)(Malate_1234/Malate) - (CS + PEP/Pyr_cycling+ SC)(OAA_1234/OAA)

dOAA_12/dt = PC(Pyruvate_12/Pyruvate) + 0.5(CS+SC)(Malate_12/Malate) + 0.5(CS+SC)(Malate_34/Malate) - (CS + PEP/Pyr_cycling+ SC)(OAA_12/OAA)

dOAA_34/dt = PC(NA_0/NA) + 0.5(CS+SC)(Malate_12/Malate) + 0.5(CS+SC)(Malate_34/Malate) - (CS + PEP/Pyr_cycling+ SC)(OAA_34/OAA)

dOAA_13/dt = PC(Pyruvate_13/Pyruvate) + 0.5(CS+SC)(Malate_13/Malate) + 0.5(CS+SC)(Malate_24/Malate) - (CS + PEP/Pyr_cycling+ SC)(OAA_13/OAA)

dOAA_14/dt = PC(NA_0/NA) + (CS+SC)(Malate_14/Malate) - (CS + PEP/Pyr_cycling+ SC)(OAA_14/OAA)

dOAA_23/dt = PC(Pyruvate_23/Pyruvate) + (CS+SC)(Malate_23/Malate) - (CS + PEP/Pyr_cycling+ SC)(OAA_23/OAA)

dOAA_24/dt = PC(NA_0/NA) + 0.5(CS+SC)(Malate_24/Malate) + 0.5(CS+SC)(Malate_13/Malate) - (CS + PEP/Pyr_cycling+ SC)(OAA_24/OAA)

dOAA_1/dt = PC(Pyruvate_1/Pyruvate) + 0.5(CS+SC)(Malate_1/Malate) + 0.5(CS+SC)(Malate_4/Malate) - (CS + PEP/Pyr_cycling+ SC)(OAA_1/OAA)

dOAA_2/dt = PC(Pyruvate_2/Pyruvate) + 0.5(CS+SC)(Malate_2/Malate) + 0.5(CS+SC)(Malate_3/Malate) - (CS + PEP/Pyr_cycling+ SC)(OAA_2/OAA)

dOAA_3/dt = PC(Pyruvate_3/Pyruvate) + 0.5(CS+SC)(Malate_3/Malate) + 0.5(CS+SC)(Malate_2/Malate) - (CS + PEP/Pyr_cycling+ SC)(OAA_3/OAA)

dOAA_4/dt = PC(NA_0/NA) + 0.5(CS+SC)(Malate_1/Malate) + 0.5(CS+SC)(Malate_4/Malate) - (CS + PEP/Pyr_cycling+ SC)(OAA_4/OAA)

As shown by the equations above, the model generates and describes the reactions giving rise to all possible isotopomers for the considered metabolites. As target data we used the time courses of citrate, glutamate, succinate, malate and OAA enrichments (Supplemental Table S3).

As shown in Supplemental Table S3, individual isotopomers were used as target data whenever possible. In some instances, the lack position-specific labeling information from certain fragments did not allow it. Such were, for instance, the cases of succinate, malate and OAA M^{+2} enrichments. This was resolved by creating combined pools for each metabolite containing all the isotopomers with two labeled carbons.

The rates of V_{PDH}, V_{PC}, V_{ICDH} and $V_{Glut_{Dil}}$ were allowed to vary freely in order to obtain the best fit possible to these time courses. The fits of the target data from INS-1 cells incubated with 9mM [U-$^{13}C_6$]glucose are shown in Supplemental Figure S6.

Supplemental Reference:

MacDonald, M.J., Brown, L.J., Longacre, M.J., Stoker, S.W., Kendrick, M.A., and Hasan, N.M. (2013). Knockdown of both mitochondrial isocitrate dehydrogenase enzymes in pancreatic beta cells inhibits insulin secretion. Biochim. Biophys. Acta *1830*, 5104–5111.