Isotopomer Analysis

For a subset of the treatment groups (10 mM PA and untreated control) reaction fluxes were estimated using both metabolite uptake/output and isotopic label distribution data. Figure S1 summarizes the overall flux estimation scheme. The problem is initialized with a random flux distribution $\mathbf{v_0}$ that satisfies the steady-state metabolite balance constraints:

$$\mathbf{S} \cdot \mathbf{v} = \mathbf{0} \tag{S1}$$

where **S** is the stoichiometric matrix and **v** is the steady-state reaction flux vector. This initial flux estimate is then combined with the MID of the input substrate to initialize the corresponding steady-state mass isotopomer distribution (MID) of selected intracellular metabolites. In this study, the input substrate was [1,2-^{13}C]glucose at 100% enrichment. The metabolites selected for MID simulation were citrate (CIT) and malate (MAL). The inputs and target metabolites were selected based on preliminary simulations as described in the next section.

To reduce the number of isotopomer variables and equations for this simulation, we first performed an elementary metabolite unit (EMU) decomposition analysis as previously described in (Antoniewicz, et al., 2007). For brevity, we illustrate the EMU analysis for a portion of the adipocyte model around the TCA cycle. A schematic of this example sub-network is shown in Figure S2. Starting with the largest EMU of the target metabolite (in this case citrate) we record all of the EMU reactions that form the target EMU from EMUs of equivalent or smaller size. This process is repeated until the target EMU has been traced back to either the input substrate (in this case pyruvate) or EMUs formed by reactions that have already been recorded. Table S5 lists the complete set of EMU reactions for the TCA cycle sub-network in order of decreasing EMU size. The first entry in Table S5 shows the EMU reaction forming CIT123456, which is the largest possible EMU of citrate with ^{13}C labels on each of the metabolite's 6 carbon atoms. The

EMUs needed to form CIT123456 are OAA1234 and AcCoA12. The next group of reactions involves EMUs that either form OAA1234 or one of its precursors, e.g. MAL1234. Note that OAA1234 and MAL1234 have the same size. The last entry of Table S5 shows the EMU reaction forming AcCoA1 from PYR2.

Following the decomposition analysis, which is performed once, the EMU reactions are grouped into decoupled sub-networks according to the size of the product EMU. Isotopomer balances are then written for each subnetwork relating the steady-state reaction fluxes to the MIDs of the corresponding EMUs. In the TCA cycle model, CIT123456 is formed from OAA1234 and AcCoA12 via reaction 3 and further metabolized via reaction 4:

$$v_3 \cdot (OAA1234 \times AcCoA12) - v_4 \cdot CIT123456 = 0 \quad (S2)$$

Similarly, OAA1234 is related to the EMUs of PYR, CO_2 and MAL:

$$v_2 \cdot (PYR123 \times CO_2 1) + v_9 \cdot MAL1234 - v_3 \cdot OAA1234 = 0 \quad (S3)$$

The last equations written are balances involving PYR2 and AcCoA1, which are the smallest EMUs (size 1):

$$v_1 \cdot PYR2 - v_2 \cdot AcCoA1 = 0 \quad (S4)$$

The operator '×' in equations S2 and S3 is the Cauchy product; e.g. the MID of CIT123456 in equation S2 is the convolution of MID of OAA1234 and MID of AcCoA12. For the adipocyte model of this study, the total number of linear EMU balances was 243. The corresponding full isotopomer model would have required 1381 non-linear equations. The EMU isotopomer equations are combined with the stoichiometric balances (equation S1) as equality constraints to solve for a flux distribution that minimizes the sum-squared difference between the calculated and measured flux and MID values. This is a non-linear optimization problem with the following objective function:

$$\text{Min.: } (\mathbf{v}_{meas} - \mathbf{v}_{sim})^T(\mathbf{v}_{meas} - \mathbf{v}_{sim}) + (\mathbf{MID}_{meas} - \mathbf{MID}_{sim})^T(\mathbf{MID}_{meas} - \mathbf{MID}_{sim}) \quad \text{(S5)}$$

In equation S5, $\mathbf{v}_{meas}$ and $\mathbf{v}_{sim}$ refer to the vector of measured and calculated exchange fluxes and $\mathbf{MID}_{meas}$ and $\mathbf{MID}_{sim}$ refer to the matrix of measured and simulated MIDs of selected intracellular metabolites.

Input Label

A series of preliminary simulations were performed to select an appropriate labeling input substrate. The ideal input leads to high ^{13}C enrichment of intracellular metabolites and generates MIDs that are sensitive to different metabolic flux distributions. We selected glucose as the input substrate, because it is the major carbon source of cultured adipocytes and is quantitatively converted to major intermediates of both carbohydrate and lipid metabolism. We tested the following glucose isotopomers: [1-^{13}C]glucose, [1,2-^{13}C]glucose and [U-^{13}C]glucose. A fourth test case was a 50:50 mixture of [1,2-^{13}C]glucose and [U-^{13}C]glucose. In every case, it was assumed that all of the glucose in the culture medium was labeled (100% enrichment). We used the following scheme to evaluate the four isotopomers as inputs. First, a previously published reaction flux data set (Si, et al., 2007) was used to calculate a corresponding steady-state MID for citrate and glyceraldehyde 3-phosphate (GAP) (see next section for selection of target metabolites). This test flux data set was calculated from measured metabolite input and output rates for an adipocyte metabolic network model that is identical to the model of this study. Next, we solved the EMU-based flux optimization problem (equation S5) for each of the four cases with the calculated MIDs of citrate and GAP as "measurements." Finally, the flux distributions from the optimization problem were compared to the test flux data set. These comparisons showed the best accuracy (smallest fractional errors) for [1,2-^{13}C]glucose (Figure S2).

Target Metabolites for MID Analysis

For a given input, the accuracy of EMU-based flux estimation generally improved when the number of target metabolites was increased from one to three. Using MIDs of three metabolites (e.g. GAP/phospho*enol*pyruvate (PEP)/MAL or GAP/PYR/OAA), it was possible to obtain flux estimates with less than 5.1% maximal error (Figure S3, panels C and D). On the other hand, the gain in accuracy was relatively small compared to the flux estimates obtained using MIDs of two metabolites. For example, the maximal errors were 5.1% and 5.3%, respectively, with MAL/CIT and GAP/CIT (Figure S3, panels A and B). In this study, we measured the MIDs of MAL/CIT, because MAL and CIT were more robustly detected than GAP by our extraction and LC-MS protocols.

Table S1. Reaction stoichiometry of the model adipocyte network

RXN	Pathway	Stoichiometry
1	Glycolysis	**Glucose** + ATP = Glucose 6-P + ADP
2	Glycolysis	Glucose 6-P = Fructose 6-P
3	Glycolysis	Fructose 6-P + ATP = Glyceraldehyde 3-P + Glycerone-P + ADP
4	Glycolysis	Glycerone-P = Glyceraldehyde 3-P
5	Glycolysis	Glyceraldehyde 3-P + NAD+ + ADP + Pi = P-Enolpyruvate + ATP + H2O
6	Glycolysis	P-Enolpyruvate + ADP + H+ = Pyruvate + ATP
7	Glycolysis	Pyruvate + NADH + H+ = **Lactate** + NAD+
8	Pentose phosphate pathway	Glucose 6-P + 2 NADP+ + H2O = Ribulose 5-P + CO2 + 2 NADPH + 2 H+
9	Pentose phosphate pathway	3 Ribulose 5-P = 2 Fructose 6-P + Glyceraldehyde 3-P
10	TCA cycle (mitochondria)	***Pyruvate*** + ***Oxaloacetate*** + NAD+ + H2O = ***Citrate*** + CO2 + NADH + H+
11	TCA cycle (mitochondria)	***Pyruvate*** + HCO3- + ATP = ***Oxaloacetate*** + ADP + Pi
12	TCA cycle (mitochondria)	***Citrate*** + NAD+ = ***2-Oxoglutarate*** + CO2 + NADH + H+
13	TCA cycle (mitochondria)	***2-Oxoglutarate*** + NAD+ + CoA = ***Succinyl-CoA*** + CO2 + NADH
14	TCA cycle (mitochondria)	***Succinyl-CoA*** + FAD + Pi + ADP = ***Fumarate*** + FADH2 + ATP + CoA
15	TCA cycle (mitochondria)	***Fumarate*** + H2O = ***Malate***
16	TCA cycle (mitochondria)	***Malate*** + NAD+ = ***Oxaloacetate*** + NADH + H+
17	TCA cycle	Citrate + CoA + ATP = Acetyl-CoA + Oxaloacetate + ADP + Pi
18	TCA cycle	Oxaloacetate + NADH + H+ = Malate + NAD+
19	TCA cycle	Malate + NADP+ = Pyruvate + CO2 + NADPH
20	TCA cycle	Citrate + NADP+ = 2-Oxoglutarate + CO2 + NADPH + H+
21	TCA cycle	Oxaloacetate + ATP = P-Enolpyruvate + CO2 + ADP
22	Oxidative phosphorylation	NADH + 0.5 O2 + 3 ADP + 3 Pi + 4 H+ = NAD+ + 3 ATP + 4 H2O
23	Oxidative phosphorylation	FADH2 + 0.5 O2 + 2 ADP + 2 Pi + 3 H+ = FAD + 2 ATP + 3 H2O
24	Palmitate biosynthesis	8 Acetyl-CoA + 14 NADPH + 7 ATP + 7 HCO3- + 14 H+ = Palmitate + 14 NADP+ + 8 CoA + 7 ADP + 7 Pi + 7 CO2 + 6 H2O
25	Tripalmitoylglycerol accumulation	Tripalmitoylglycerol = **Tripalmitoylglycerol**
26	Tripalmitoylglycerol biosynthesis	Glycerone-P + 3 Palmitate + NADH + 3 ATP + H2O + H+ = Tripalmitoylglycerol + NAD+ + Pi + 3 AMP + 3 PPi
27	Tripalmitoylglycerol biosynthesis	Tripalmitoylglycerol + 3 H2O = **Glycerol** + 3 Palmitate
28	Metabolism of ketone bodies	2 ***Acetyl-CoA*** = ***Acetoacetate*** + 2 CoA
29	Metabolism of ketone bodies	***Acetoacetyl-CoA*** = ***Acetoacetate*** + CoA
30	Metabolism of ketone bodies	***Acetoacetate*** + NADH = **3-Hydroxybutyrate**
31	Amino acid metabolism	Pyruvate + NH4+ + NADPH = Alanine
32	Amino acid metabolism	Aspartate + NH4+ = **Asparagine**
33	Amino acid metabolism	Aspartate = Oxaloacetate + NH4+ + NADH
34	Amino acid metabolism	Cysteine = Pyruvate + NH4+ + NADH
35	Amino acid metabolism	Glutamate = 2-Oxoglutarate + NH4+ + NADH

36	Amino acid metabolism	Glutamate + NH4+ + ATP = **Glutamine**
37	Amino acid metabolism	Serine + THF = Glycine
38	Amino acid metabolism	**Histidine** + THF = Glutamate + NH4+
39	Amino acid metabolism	**Isoleucine** + 2 CoA = ***Succinyl-CoA*** + ***Acetyl-CoA*** + NH4+ + FADH2 + 2 NADH
40	Amino acid metabolism	**Leucine** + CoA + CO2 + ATP = ***Acetoacetate*** + ***Acetyl-CoA*** + NH4+ + FADH2 + 2 NADH
41	Amino acid metabolism	**Lysine** = 2-Oxoadipate + 2 NH4+ + 3 NADH
42	Amino acid metabolism	2-Oxoadipate + CoA = ***Acetoacetyl-CoA*** + 2 CO2 + FADH2 + 2 NADH
43	Amino acid metabolism	**Methionine** + Serine + ATP + CoA + THF = ***Succinyl-CoA*** + Cysteine + NH4+ + NADH
44	Amino acid metabolism	**Phenylalanine** + O2 + NADH = Tyrosine
45	Amino acid metabolism	Glutamate + ATP + 2 NADPH = **Proline**
46	Amino acid metabolism	Serine = Pyruvate + NH4+
47	Amino acid metabolism	**Threonine** + CoA = Glycine + ***Acetyl-CoA*** + NADH
48	Amino acid metabolism	**Tryptophan** + 3 O2 + NADPH = 2-Oxoadipate + Alanine + CO2 + NH4+
49	Amino acid metabolism	Tyrosine + 2 O2 = ***Acetoacetate*** + ***Fumarate*** + CO2 + NH4+ + NADH
50	Amino acid metabolism	**Valine** + CoA = ***Succinyl-CoA*** + CO2 + 4 NADH + FADH2 + NH4+
51	Plasma exchange	Palmitate = **Palmitate**
52	Plasma exchange	***Acetoacetate*** = **Acetoacetate**
53	Plasma exchange	Alanine = **Alanine**
54	Plasma exchange	**Aspartate** = Aspartate
55	Plasma exchange	**Cysteine** = Cysteine
56	Plasma exchange	**Glutamate =** Glutamate
57	Plasma exchange	Glycine = **Glycine**
58	Plasma exchange	**Serine** = Serine
59	Plasma exchange	**Tyrosine** = Tyrosine
60	Plasma exchange	**O2 =** O2
61	Plasma exchange	CO2 = **CO2**
62	Plasma exchange	NH4+ = **NH4+**
63	Mitochondrial exchange	Pyruvate = ***Pyruvate***
64	Mitochondrial exchange	***Citrate*** + Malate = Citrate + ***Malate***
65	Mitochondrial exchange	2-Oxoglutarate + ***Malate*** = ***2-Oxoglutarate*** + Malate
66	Mitochondrial exchange	***Malate*** + Pi = Malate + ***Pi***

Extracellular metabolites are indicated in bold. Mitochondrial metabolites are indicated in bold italics. Several entries in the table (e.g. reaction #10) represent pseudo-reactions obtained by condensing sequences of non-branching reactions.

Table S2. Amino acid uptake or output on day 12 post-induction

Amino Acid	Control	20 mM OXA		5 mM PA		10 mM PA	
ALA	0.7 ± 0.0	0.6 ± 0.0	*	0.4 ± 0.1	*	-0.5 ± 0.1	*
ASN	-3.0 ± 0.1	-1.1 ± 0.2	*	-4.6 ± 0.2	*	-5.9 ± 0.4	*
ASP	0.0 ± 0.0	0.0 ± 0.0		0.0 ± 0.0		0.0 ± 0.0	
GLN	-11.2 ± 1.4	-12.0 ± 1.5		-7.9 ± 0.6	*	2.6 ± 2.9	*
GLU	0.4 ± 0.0	0.4 ± 0.0	*	0.3 ± 0.0	*	0.3 ± 0.0	*
GLY	-8.8 ± 0.4	-5.9 ± 0.4	*	-7.3 ± 0.2	*	-7.9 ± 0.9	
HIS	-0.4 ± 0.1	-0.2 ± 0.1	*	-0.1 ± 0.1	*	-0.1 ± 0.2	*
ILE	8.2 ± 0.2	6.1 ± 0.3	*	8.1 ± 0.3		7.5 ± 0.2	*
LEU	8.8 ± 0.2	6.8 ± 0.3	*	8.6 ± 0.3		7.9 ± 0.2	*
LYS	0.3 ± 0.5	-2.0 ± 0.3	*	-1.8 ± 0.1	*	-2.0 ± 0.2	*
MET	0.1 ± 0.1	0.0 ± 0.0		0.1 ± 0.1		0.1 ± 0.2	
PHE	-0.8 ± 0.1	-0.3 ± 0.1	*	-0.1 ± 0.1	*	-0.1 ± 0.4	*
PRO	-0.4 ± 0.1	-0.5 ± 0.0		-1.4 ± 0.0	*	-2.1 ± 0.2	*
SER	4.1 ± 0.1	4.0 ± 0.2		4.5 ± 0.2	*	4.6 ± 0.5	
THR	-1.0 ± 0.3	-1.4 ± 0.3		-1.2 ± 0.1		-1.4 ± 0.4	
TYR	-0.7 ± 0.1	-0.3 ± 0.1	*	0.1 ± 0.2	*	-0.0 ± 0.4	*
VAL	5.5 ± 0.2	3.3 ± 0.2	*	5.1 ± 0.4		3.8 ± 0.4	*

All units are in mmol/g-DNA/2 days. Data shown are means ± SD (n = 6). *: statistically significantly different from control ($p < 0.05$). Positive value indicates uptake.

Table S3. Metabolic flux profiles on day 12 post-induction

RXN	Control	OXA 20 mM		PA 5 mM		PA 10 mM	
1	266.0 ± 16.2	212.3 ± 15.3	*	367.0 ± 17.6	*	341.0 ± 14.4	*
2	225.3 ± 12.9	205.0 ± 14.2	*	359.9 ± 23.3	*	316.7 ± 19.4	*
3	252.4 ± 13.4	209.9 ± 14.8	*	364.7 ± 19.3	*	332.9 ± 13.7	*
4	239.0 ± 12.8	187.8 ± 13.9	*	342.7 ± 18.4	*	309.4 ± 13.0	*
5	505.1 ± 28.5	400.1 ± 28.6	*	709.8 ± 35.8	*	650.5 ± 26.0	*
6	629.4 ± 47.6	463.5 ± 35.6	*	784.6 ± 35.5	*	725.6 ± 35.8	*
7	200.0 ± 23.5	119.2 ± 9.1	*	410.4 ± 19.1	*	427.8 ± 9.4	*
8	40.7 ± 14.9	7.3 ± 4.6	*	7.1 ± 7.8	*	24.3 ± 18.5	
9	13.6 ± 5.0	2.4 ± 1.5	*	2.4 ± 2.6	*	8.1 ± 6.2	
10	318.7 ± 41.3	290.4 ± 25.6		312.7 ± 48.2		233.7 ± 25.1	*
11	184.6 ± 26.1	109.1 ± 31.7	*	141.4 ± 13.2	*	123.5 ± 15.3	*
12	132.2 ± 32.6	185.0 ± 19.7	*	200.9 ± 54.1	*	132.2 ± 19.3	
13	194.4 ± 32.7	226.7 ± 26.2		237.8 ± 52.0		158.6 ± 26.1	
14	208.1 ± 32.6	236.0 ± 26.3		251.0 ± 52.6		169.9 ± 26.6	
15	208.1 ± 32.6	236.0 ± 26.3		251.2 ± 52.7		170.1 ± 27.0	
16	134.2 ± 34.7	181.2 ± 40.8		171.3 ± 45.2		110.2 ± 17.8	
17	124.4 ± 21.8	63.7 ± 12.9	*	74.9 ± 9.0	*	75.1 ± 16.4	*
18	0.1 ± 0.1	0.3 ± 0.8		0.0 ± 0.0		0.0 ± 0.0	
19	74.0 ± 12.1	55.2 ± 20.8		79.9 ± 9.7		59.9 ± 9.2	*
20	62.2 ± 20.0	41.6 ± 9.1		36.9 ± 7.3	*	26.4 ± 6.9	*
21	124.3 ± 21.9	63.4 ± 12.9	*	74.8 ± 9.0	*	75.1 ± 16.4	*
22	607.9 ± 150.0	770.4 ± 108.2		527.6 ± 214.3		213.9 ± 89.2	*
23	230.8 ± 32.6	252.2 ± 26.4		272.8 ± 53.3		189.1 ± 27.3	*
24	15.5 ± 2.7	8.0 ± 1.6	*	9.4 ± 1.1	*	9.4 ± 2.0	*
25	13.4 ± 1.4	22.1 ± 4.8	*	22.0 ± 1.3	*	23.5 ± 3.0	*
26	8.6 ± 0.8	19.8 ± 4.4	*	19.2 ± 1.4	*	20.6 ± 2.4	*
27	4.8 ± 0.9	2.3 ± 0.5	*	2.8 ± 0.4	*	2.9 ± 0.6	*
28	8.9 ± 0.2	6.4 ± 0.3	*	8.4 ± 0.3	*	7.8 ± 0.2	*
29	0.3 ± 0.4	0.0 ± 0.0		0.0 ± 0.0		0.0 ± 0.0	
30	16.5 ± 1.3	10.8 ± 2.2	*	17.0 ± 0.6		15.9 ± 0.7	
31	0.0 ± 0.0	0.0 ± 0.0		0.0 ± 0.0		0.5 ± 0.1	*
32	1.5 ± 0.1	0.6 ± 0.1	*	2.3 ± 0.1	*	2.9 ± 0.2	*
33	0.0 ± 0.0	0.0 ± 0.0		0.0 ± 0.0		0.0 ± 0.0	
34	0.0 ± 0.0	0.0 ± 0.0		0.0 ± 0.0		0.0 ± 0.0	
35	0.0 ± 0.0	0.0 ± 0.0		0.0 ± 0.0		0.0 ± 0.0	*
36	7.5 ± 0.9	8.0 ± 1.0		5.3 ± 0.4	*	0.0 ± 0.0	*
37	6.0 ± 0.3	5.0 ± 0.3	*	5.8 ± 0.2		6.2 ± 0.4	
38	3.8 ± 0.5	4.1 ± 0.5		2.7 ± 0.2	*	0.5 ± 0.1	*
39	8.2 ± 0.2	6.1 ± 0.3	*	8.1 ± 0.3		7.5 ± 0.2	*
40	8.8 ± 0.2	6.8 ± 0.3	*	8.6 ± 0.3		7.9 ± 0.2	*
41	0.3 ± 0.4	0.0 ± 0.0		0.0 ± 0.0		0.0 ± 0.0	
42	0.3 ± 0.4	0.0 ± 0.0		0.0 ± 0.0		0.0 ± 0.0	
43	0.0 ± 0.0	0.0 ± 0.0		0.0 ± 0.0		0.0 ± 0.0	
44	0.0 ± 0.0	0.0 ± 0.0		0.0 ± 0.0		0.1 ± 0.3	
45	0.0 ± 0.0	0.0 ± 0.0	*	0.0 ± 0.0		1.5 ± 0.1	*
46	0.0 ± 0.0	0.0 ± 0.0		0.0 ± 0.0		0.0 ± 0.0	
47	0.9 ± 0.1	0.0 ± 0.0	*	0.1 ± 0.1	*	0.2 ± 0.2	*

48	0.0	±	0.0	0.0	±	0.0		0.0	±	0.0		0.0	±	0.0	
49	0.0	±	0.0	0.0	±	0.0		0.2	±	0.1		0.2	±	0.5	
50	5.5	±	0.2	3.3	±	0.2	*	5.1	±	0.4		3.8	±	0.4	*
51	1.1	±	0.1	1.0	±	0.1		1.0	±	0.1	*	0.8	±	0.1	*
52	1.5	±	1.2	2.4	±	1.9		0.2	±	0.2	*	0.0	±	0.1	*
53	0.0	±	0.0	0.0	±	0.0		0.0	±	0.0		0.5	±	0.1	*
54	1.5	±	0.1	0.6	±	0.1	*	2.3	±	0.1	*	2.9	±	0.2	*
55	0.0	±	0.0	0.0	±	0.0		0.0	±	0.0		0.0	±	0.0	
56	3.6	±	0.4	3.9	±	0.5		2.6	±	0.2	*	0.9	±	0.1	*
57	6.9	±	0.2	5.0	±	0.3	*	5.9	±	0.2	*	6.4	±	0.4	*
58	6.0	±	0.3	5.0	±	0.3	*	5.8	±	0.2		6.2	±	0.4	
59	0.0	±	0.0	0.0	±	0.0		0.1	±	0.1	*	0.1	±	0.2	
60	419.3	±	91.2	511.3	±	67.1		400.5	±	134.0		202.1	±	59.1	*
61	759.2	±	105.9	756.9	±	75.2		805.5	±	145.9		582.8	±	66.5	*
62	17.9	±	1.7	11.7	±	0.5	*	17.0	±	1.0		16.5	±	1.3	
63	503.3	±	59.8	399.5	±	40.8	*	454.0	±	54.4		357.2	±	37.5	*
64	186.6	±	39.0	105.3	±	13.9	*	111.7	±	15.8	*	101.5	±	16.0	*
65	62.2	±	20.0	41.6	±	9.1		36.9	±	7.3	*	26.4	±	6.9	*
66	73.9	±	12.1	54.8	±	20.5		79.9	±	9.7		59.9	±	9.2	

All units are in mmol/g-DNA/2 days. Data shown are means ± SD (n = 6). *: statistically significantly different from untreated control ($p < 0.05$). Reaction numbers refer to Supplementary Table 1.

Table S4. Comparison of flux estimates from stoichiometric and isotopomer model

RXN	Control				10 mM PA			
	Stoichiometric model		Isotopomer analysis		Stoichiometric model		Isotopomer analysis	
1	100.0 ± 11.6		99.5 ± 19.9		87.9 ± 4.8		86.3 ± 6.7	
2	71.9 ± 14.8		74.6 ± 13.7		73.3 ± 7.7		76.8 ± 10.8	
3	90.6 ± 12.6		91.2 ± 17.5		83.0 ± 4.4		83.1 ± 8.0	
4	85.2 ± 12.3		82.7 ± 22.1		77.1 ± 3.7		76.3 ± 5.5	
5	185.1 ± 23.9		182.2 ± 42.0		165.0 ± 7.8		162.5 ± 12.1	
6	234.1 ± 30.8		209.8 ± 60.7		175.2 ± 11.9		165.7 ± 9.5	
7	70.1 ± 5.5		70.3 ± 9.8		129.2 ± 8.3		130.4 ± 7.3	
8	28.1 ± 4.1		24.9 ± 9.8		14.6 ± 8.3		9.5 ± 4.3	
9	9.4 ± 1.4		8.3 ± 3.3		4.9 ± 2.8		3.2 ± 1.4	
10	121.7 ± 21.8		126.5 ± 59.5		42.8 ± 6.3		34.2 ± 5.7	
11	69.4 ± 15.4		105.6 ± 65.9		11.1 ± 9.1		1.7 ± 4.5	
12	71.7 ± 14.2		3.4 ± 13.0		21.8 ± 3.5		23.8 ± 15.1	
13	72.7 ± 18.7		29.8 ± 7.4		26.0 ± 3.5		25.8 ± 12.5	
14	77.7 ± 18.6		39.2 ± 9.2		31.7 ± 4.0		32.5 ± 8.2	
15	77.7 ± 18.8		39.3 ± 11.8		31.7 ± 4.2		32.5 ± 10.2	
16	52.4 ± 7.5		20.9 ± 15.3		31.7 ± 3.7		32.5 ± 10.2	
17	49.0 ± 3.2		96.8 ± 54.7		19.4 ± 8.9		10.2 ± 10.5	
18	0.0 ± 0.0		71.3 ± 43.6		9.2 ± 0.0		7.1 ± 6.0	
19	25.4 ± 8.3		89.7 ± 54.8		9.2 ± 1.5		7.1 ± 6.0	
20	1.1 ± 4.4		26.4 ± 17.9		1.5 ± 0.0		0.1 ± 0.0	
21	49.0 ± 7.3		27.6 ± 21.0		10.2 ± 8.9		3.1 ± 3.9	
22	258.4 ± 64.0		60.6 ± 12.7		0.0 ± 0.0		0.0 ± 0.0	
23	85.8 ± 18.5		50.0 ± 8.1		39.7 ± 4.5		41.5 ± 1.9	
24	6.1 ± 0.4		12.1 ± 6.8		2.4 ± 1.1		1.3 ± 1.3	
25	2.1 ± 0.3		4.0 ± 5.5		0.8 ± 0.4		0.7 ± 0.2	
26	5.5 ± 0.2		8.5 ± 6.9		5.9 ± 1.1		6.9 ± 3.0	
27	3.4 ± 0.0		4.5 ± 0.0		5.1 ± 0.9		6.2 ± 3.0	
28	3.1 ± 0.2		3.5 ± 1.9		3.6 ± 0.0		4.1 ± 0.1	
29	0.0 ± 0.0		0.0 ± 0.0		0.0 ± 0.0		0.0 ± 0.0	
30	4.8 ± 0.0		5.0 ± 6.6		4.0 ± 1.1		4.9 ± 1.8	
31	0.4 ± 0.1		0.5 ± 2.3		3.7 ± 0.3		7.2 ± 1.9	
32	0.4 ± 0.1		-0.6 ± 1.8		0.9 ± 0.0		1.4 ± 0.6	
33	0.0 ± 0.0		2.1 ± 0.7		0.0 ± 0.0		0.0 ± 0.0	
34	0.7 ± 0.0		2.5 ± 0.0		2.4 ± 0.0		0.7 ± 0.1	
35	0.0 ± 0.0		0.0 ± 0.0		2.6 ± 0.5		1.9 ± 5.2	
36	9.3 ± 0.0		9.3 ± 0.0		-3.2 ± 0.4		-3.3 ± 0.8	
37	0.7 ± 0.3		0.3 ± 0.7		2.2 ± 0.0		1.1 ± 0.1	
38	4.8 ± 0.1		4.8 ± 1.4		0.0 ± 0.2		0.0 ± 1.9	

39	3.1	±	0.1	4.1	±	1.7	3.6	±	0.2	3.8	±	1.3
40	3.1	±	0.1	2.9	±	3.9	2.4	±	0.2	3.2	±	0.2
41	-0.2	±	0.2	-0.2	±	1.9	-0.1	±	0.3	1.8	±	5.4
42	0.0	±	0.0	0.0	±	1.1	0.0	±	0.4	0.0	±	0.0
43	0.0	±	0.0	1.6	±	1.4	0.1	±	0.1	0.8	±	0.6
44	-0.3	±	0.1	-0.3	±	0.2	-0.3	±	0.2	-0.7	±	1.4
45	-1.9	±	0.0	-2.0	±	0.0	1.0	±	0.2	1.5	±	2.6
46	1.4	±	0.2	0.9	±	0.3	0.0	±	0.0	0.0	±	0.0
47	0.1	±	0.0	0.0	±	1.9	1.2	±	0.2	1.1	±	1.1
48	0.2	±	0.2	0.2	±	0.3	0.1	±	0.2	-1.8	±	0.7
49	0.0	±	0.0	0.1	±	1.0	0.0	±	0.4	0.0	±	0.0
50	1.9	±	0.1	3.7	±	2.8	1.9	±	0.6	2.1	±	3.7
51	-0.1	±	0.3	0.2	±	0.6	-0.1	±	0.1	-0.8	±	1.4
52	1.3	±	0.0	1.5	±	4.7	2.0	±	0.3	2.3	±	1.9
53	0.7	±	0.0	0.7	±	0.5	3.8	±	0.2	5.4	±	1.4
54	0.4	±	0.0	1.5	±	0.4	0.9	±	0.0	1.4	±	0.3
55	0.0	±	0.0	0.6	±	0.5	0.0	±	0.1	-1.1	±	0.5
56	2.6	±	0.2	2.5	±	0.3	0.5	±	0.2	0.1	±	0.6
57	0.1	±	0.0	0.0	±	0.0	1.2	±	0.2	1.1	±	1.1
58	2.1	±	0.1	2.8	±	1.9	2.4	±	0.1	1.8	±	0.6
59	0.3	±	41.7	0.4	±	22.2	0.3	±	0.2	0.7	±	0.8
60	172.5	±	56.0	55.7	±	87.0	19.8	±	3.2	14.6	±	6.5
61	299.3	±	0.0	223.7	±	2.2	114.6	±	6.7	99.0	±	14.3
62	4.5	±	37.0	13.5	±	124.6	11.7	±	2.5	9.0	±	17.0
63	191.1	±	7.5	232.1	±	72.2	53.8	±	15.2	35.9	±	1.6
64	50.1	±	4.4	123.2	±	17.9	21.0	±	8.9	10.3	±	10.3
65	1.1	±	12.7	26.4	±	23.9	4.2	±	0.5	2.0	±	5.5
66	25.4	±	0.1	18.4	±	1.4	0.0	±	0.0	0.0	±	0.0

Flux estimates reflect metabolite and isotopomer data collected for a separate batch of cells on day 12 post-induction. Data were normalized to the glucose uptake rate (RXN #1) of the untreated control condition (100 %). Data shown are means ± SD (n = 3). Reaction numbers refer to Table S1.

Table S5. EMU reactions of the TCA cycle

Reaction No.	EMU reaction	EMU size
3	OAA1234 + AcCoA12 = CIT123456	6
8	FUM1234 = MAL1234	4
7	SUC1234 = FUM1234	4
6	SucCoA1234 = SUC1234	4
5	aKG2345 = SucCoA1234	4
4	CIT2345 = aKG2345	4
3	OAA23 + AcCoA12 = CIT2345	4
9	MAL1234 = OAA1234	4
2	PYR123 + CO21 = OAA1234	4
9	MAL23 = OAA23	2
2	PYR23 = OAA23	2
8	FUM23 = MAL23	2
7	SUC23 = FUM23	2
6	SucCoA23 = SUC23	2
5	aKG34 = SucCoA23	2
4	CIT34 = aKG34	2
3	OAA2 + AcCoA2 = CIT34	2
1	PYR3 = AcCoA2	1
2	PYR2 = OAA2	1
9	MAL2= OAA2	1
8	FUM2 = MAL2	1
7	SUC2 = FUM2	1
6	SucCoA2 = SUC2	1
6	SucCoA3 = SUC2	1
5	aKG3 = SucCoA2	1
5	aKG4 = SucCoA3	1
4	CIT3 = aKG3	1
4	CIT4 = aKG4	1
3	OAA2 = CIT3	1
3	AcCoA2 = CIT4	1
1	PYR1 = CO21	1
4	CIT6 = CO21	1
5	aKG1 = CO21	1
3	OAA1 = CIT6	1
2	PYR1 = OAA1	1
9	MAL1 = OAA1	1
8	FUM1 = MAL1	1
7	SUC1 = FUM1	1
6	SucCoA1 = SUC1	1
6	SucCoA4 = SUC1	1
5	aKG2 = SucCoA1	1
5	aKG5 = SucCoA4	1

4	CIT2 = aKG2	1
4	CIT5 = aKG5	1
4	CIT1 = aKG1	1
3	OAA4 = CIT1	1
2	CO21 = OAA4	1
9	MAL4 = OAA4	1
8	FUM4 = MAL4	1
7	SUC4 = FUM4	1
6	SucCoA4 = SUC4	1
6	SucCoA1 = SUC4	1
3	OAA3 = CIT2	1
2	PYR3 = OAA3	1
9	MAL3 = OAA3	1
8	FUM3 = MAL3	1
7	SUC3 = FUM3	1
6	SucCoA2 = SUC3	1
6	SucCoA3 = SUC3	1
3	AcCoA1 = CIT5	1
1	PYR2 = AcCoA1	1

Supplementary Figures

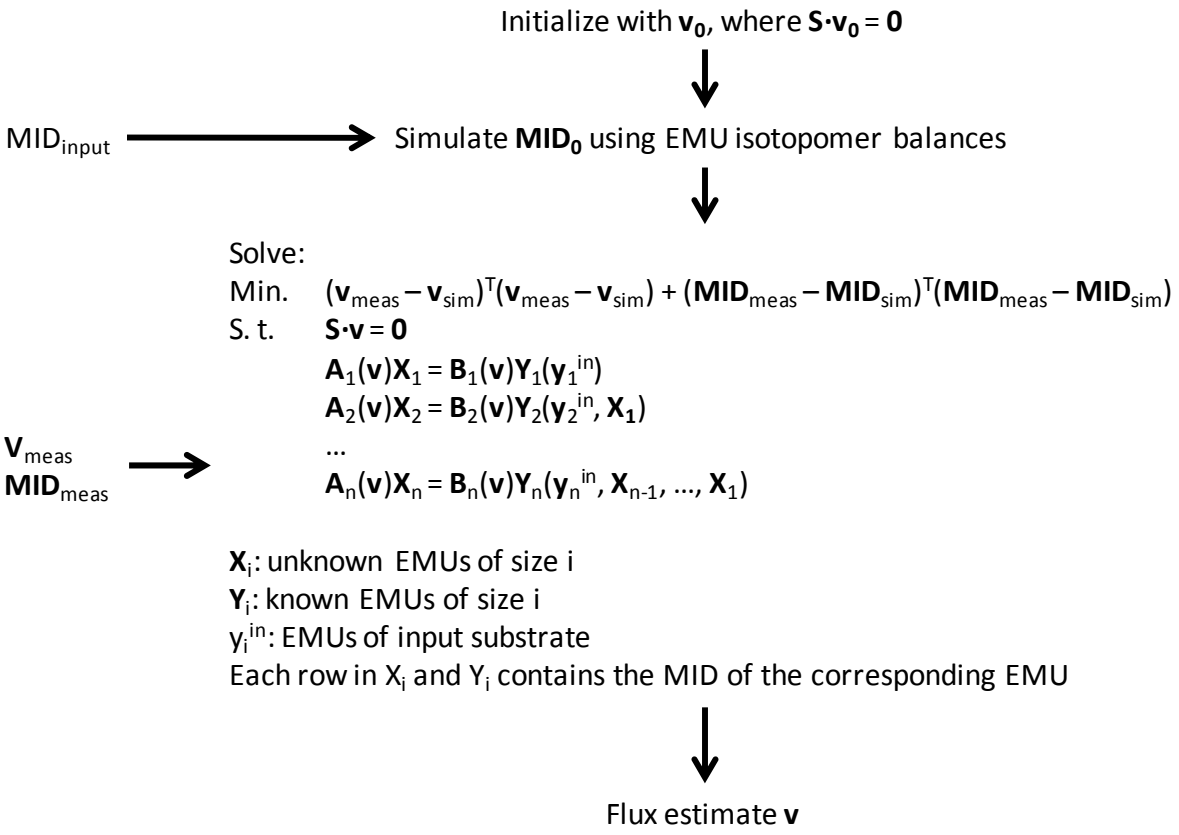

Figure S1. Schematic overview of MID-based flux calculation.

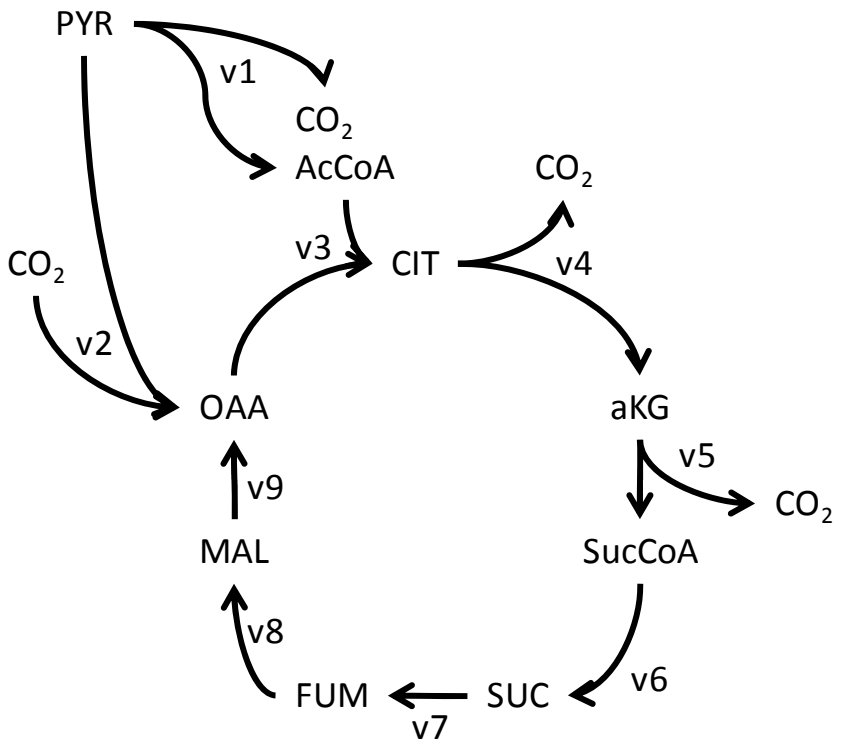

Figure S2. Example sub-network around the TCA cycle. PYR: pyruvate; AcCoA: acetyl CoA; OAA: oxaloacetate; CIT: citrate; aKG: 2-oxoglutarate; SucCoA: succinyl-CoA; SUC: succinate; FUM: fumarate; MAL: malate.

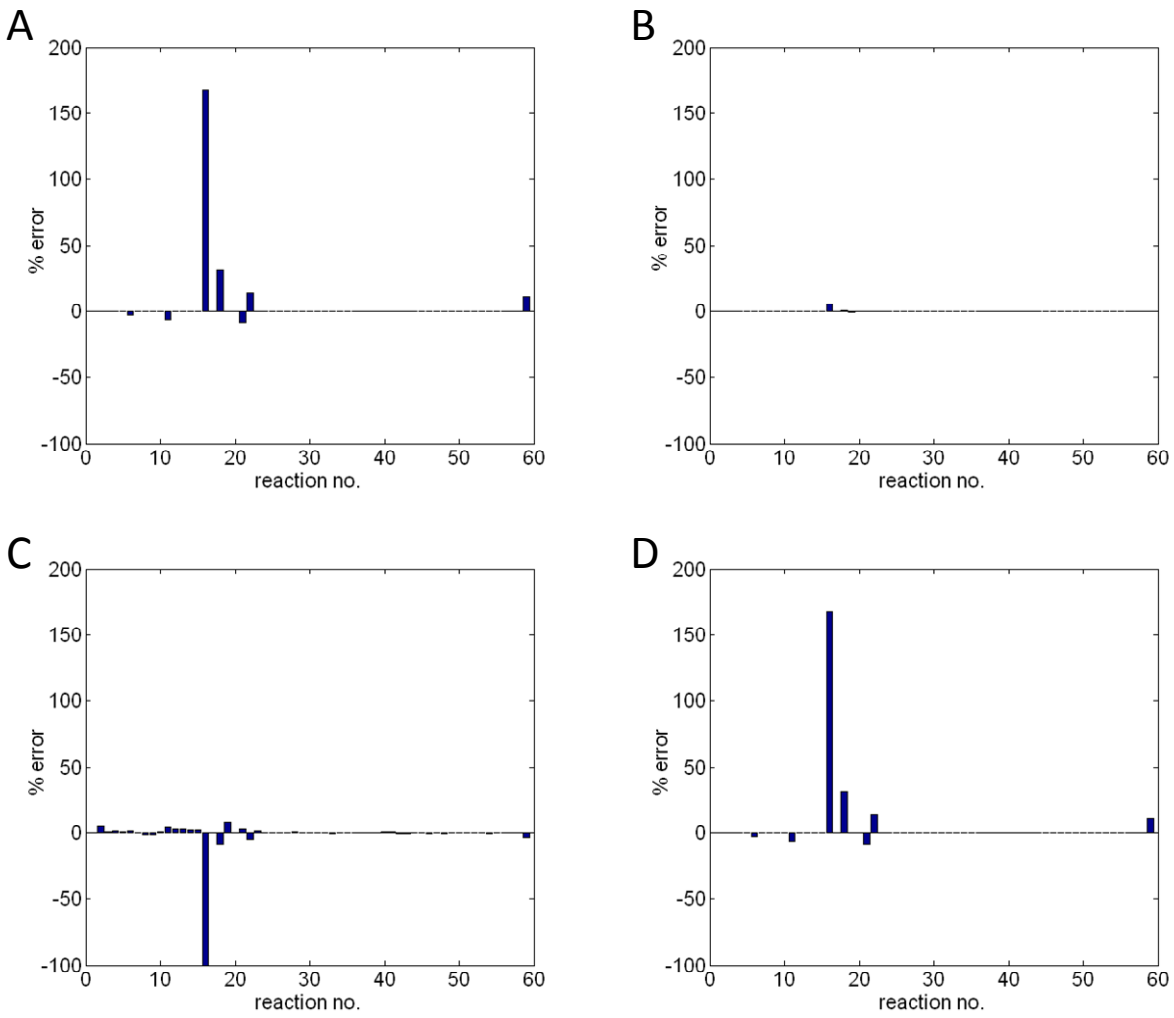

Figure S3. Simulations showing the effects of different input label choices on the accuracy of the flux estimates. Panels show the % differences between *a priori* known and MID-based flux estimates. A priori known fluxes were taken from a previous publication (see text). (A) [1-^{13}C]glucose; (B) [1,2-^{13}C]glucose; (C) [U-^{13}C]glucose; and (D) 50:50 mixture of [1,2-^{13}C]glucose and [U-^{13}C]glucose. The flux estimates are based on the simulated MIDs of GAP and CIT.

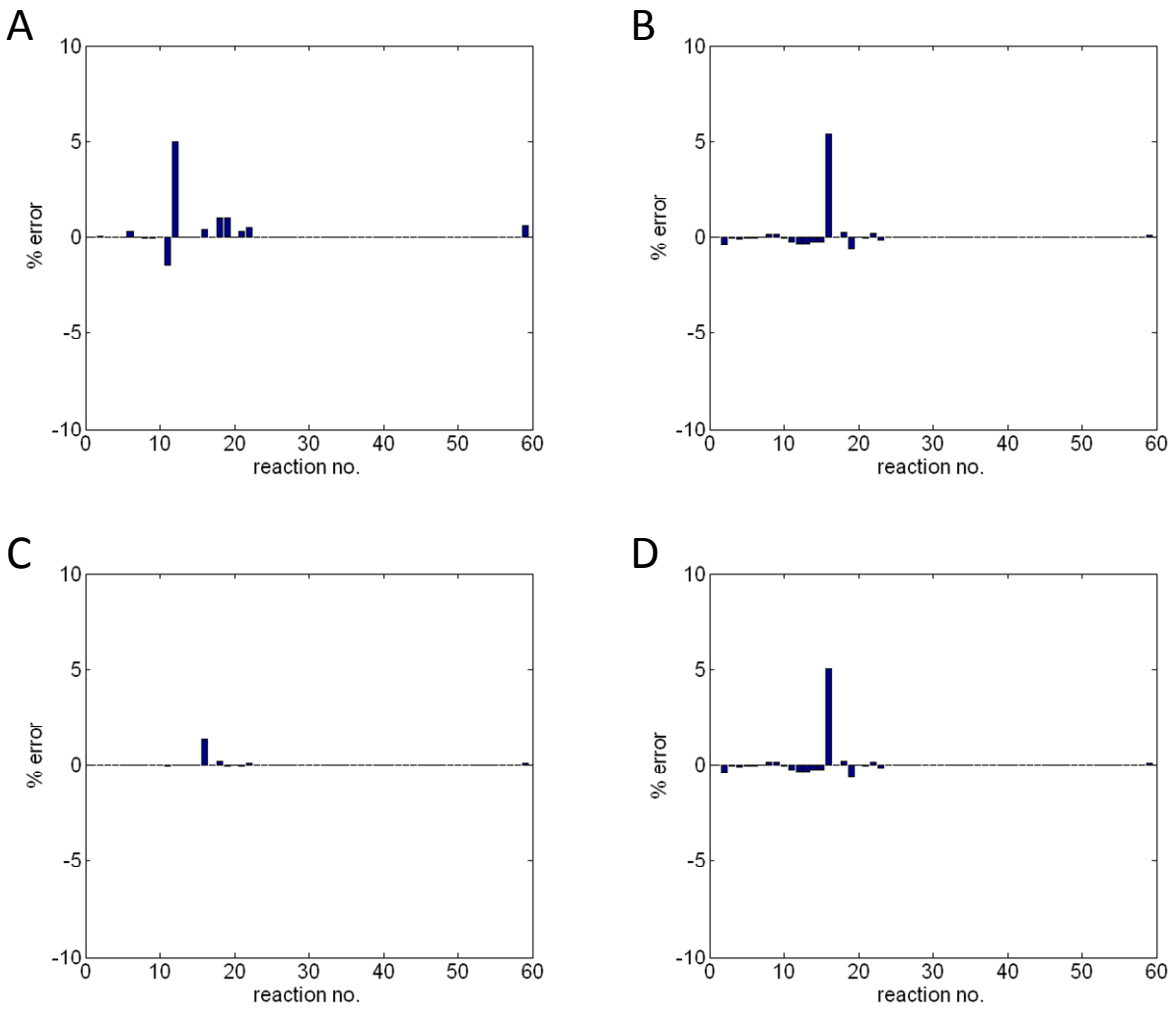

Figure S4. Simulations showing the effects of different metabolite MID choices on the accuracy of the flux estimates. Panels show the % differences between *a priori* known and MID-based flux estimates. (A) MAL and CIT; (B) CIT and GAP; (C) GAP, PEP and MAL; (D) GAP, OAA and PYR. All estimates used [1,2-^{13}C]glucose as the input label.