SUPPLEMENT

Simultaneous tracers and a unified model of positional and mass isotopomers for quantification of metabolic flux in liver

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SUPPLEMENTARY RESULTS

Constraining body water ^2H enrichment improves NMR but not GC-MS model-regressed flux estimates

The ^2H enrichment of glucose is a function of precursor, or body water (enrichment of ^2H_2O). Thus, a measurement of ^2H_2O body water enrichment can be used as an additional constraint when fitting NMR or GC-MS isotopomer data to models of hepatic metabolism using MFA. The ^2H_2O enrichment in plasma was measured by ^2H NMR using the natural ^2H abundance of acetone as a reference (**Figure S4A**) as previously reported (Jones et al., 2001a), and could be quantify enrichments as low as 0.1% (**Figure S4B**). All plasma samples were found to be close to 4% (**Figure S4D**), nearly identical to the targeted body water enrichment based on ^2H_2O injection. Though not measured, perfused livers must have a ^2H_2O enrichment of 3% based on volumes used to prepare the media (**Figure S4C**). In either case, this information improved the regression of NMR data, but not GC-MS data. The discrepancy may be related to the total lack of absolute enrichment information in the ^2H NMR spectrum of glucose, but precise information about absolute enrichment (e.g., M+0 versus M+1) in the mass spectra of glucose fragments. When body water ^2H enrichment was left as a free parameter that could be adjusted during model-based regression of GC-MS data, the predicted body ^2H_2O enrichment was close to the assumed (**Figure S4C**) or measured (**Figure S4D**) values. Thus, constraining the model to exact ^2H enrichment adds information for fitting NMR data, but apparently over-constrains GC-MS data.

Testing the agreement of NMR and GC-MS flux estimates

Every measurement implies some degree of error thus, when two independent methods are used to measure the same variable, neither may provide an unequivocally correct answer. Correlation analysis was used to assess the relationship between flux values obtained from NMR and GC-MS measurements (main text and **Figure 7**). Passing-Bablok (PB) regression and Bland Altman (BA) analysis were also used to estimate the degree of agreement between the two methods of flux quantification.

Passing-Bablok regression of flux estimates

PB regression is a nonparametric method of fitting a straight line to a set of paired variables (x and y). PB does not assume measurement error to be normally distributed and is robust against outliers. To overcome the influence of outliers, the method estimates the slope by calculating the median of all slopes that can be formed from all possible pairs; thus, it reports slope and intercept values that can differ from the results obtained using standard least-squares regression analysis (**Table S4**). We used PB regression to determine the existence and type of differences between NMR and GC-MS based flux estimates by using following criteria:

1. Does the slope confidence interval (CI) enclose the value of 1? If no, there is evidence for proportional differences between the analyzed methods.
2. Does the intercept CI enclose the value of 0? If no, there is evidence for constant differences (bias) between the analyzed methods.
3. The PB procedure is valid only when flux values obtained using NMR and GC-MS showed a linear relationship.

Indeed, PB regression indicated the presence of either constant or proportional bias in all fluxes but $V_{CS,}$ which had nearly identical values by both methods (**Table S4**). The $V_{Glycerol}$ flux could not be analyzed since it did not meet the requirement of linearity (**Figure 7**, **Table S4**). $V_{Glycogen}$ showed evidence for proportional differences, while V_{PEP}, V_{PEPCK} and V_{PK+ME} showed

both evidence for proportional and constant differences between methods (**Table S4**). These results suggest that the variance of measurement error is not constant over the range of studied flux values, and the variability increases with the magnitude of the flux estimates.

Bland-Altman analysis of flux estimates

Unlike PB regression, which only examines the existence of differences, BA analysis quantifies the magnitude of differences between the methods. In BA analysis, the difference or the mean % difference of the two paired measurements is plotted against the mean of the two measurements (**Figure S5**). BA analysis assumes the mean of the methods is correct and reports bias and range of agreement within 95%, but does not determine if the agreement is sufficient. Therefore, we used BA analysis to quantify bias for the fluxes that were flagged by PB regression.

We found that $V_{Glycogen}$ was negatively and proportionally different (-45.8%) between NMR and GC-MS methods (**Figure S5**). Indeed, both methods detected glycogen depletion; however, they differed when glycogen was a large contributor to EGP. The relatively large % bias was partially driven by differences between glycogen-depleted samples (where small absolute differences resulted in large % differences). Thus, either GC-MS overestimated, or NMR underestimated glycogen contribution to EGP. However, the latter seems rather unlikely, due to perfect distinction between positions H2 and H5 in the NMR spectrum of MAG. Therefore, we concluded that GC-MS reports slightly higher glycogen contribution (as discussed in the main text).

On the other hand, V_{PEP} (+9.4%), V_{PEPCK} (+16.7%) and V_{PK+ME} (+24.1%) showed positive constant and proportional differences for NMR compared to GC-MS (**Table S4**). Interestingly, all three of these fluxes rely strongly on the presence of a distinct quartet isotopomer in the ^{13}C NMR spectrum. However, GC-MS estimation of these fluxes requires deconvolution of many

overlapping mass isotopomers, which could lead to greater variability. The variation between NMR and GC-MS in estimates of cataplerosis/anaplerosis may be related to differences in estimates of ^{13}C M+3 isotopomers (i.e., [1,2,3-$^{13}C_3$]glucose and [4,5,6-$^{13}C_3$]glucose) or ^{2}H M+1 isotopomers. The latter is likely, inasmuch as GC-MS would estimate lower V_{PEP} due to a higher estimation of $V_{Glycogen}$ based on ^{2}H isotopomers. Such a shift in the contribution between glycogen and PEP would also affect V_{PEPCK} and V_{PK+ME} since all these fluxes are tightly connected by stoichiometric relationships. Finally, V_{CS} showed no differences in PB regression; thus, we consider the methods to agree on this flux, although BA quantified a slight (-13.6%) difference between NMR and GC-MS.

In conclusion, although both methods correlate well, they report different flux values. These biases should be taken into account when comparing studies where fluxes were obtained using NMR and GC-MS. However, when a single method is used, we expect that it will detect changes in fluxes precisely.

Figure S1

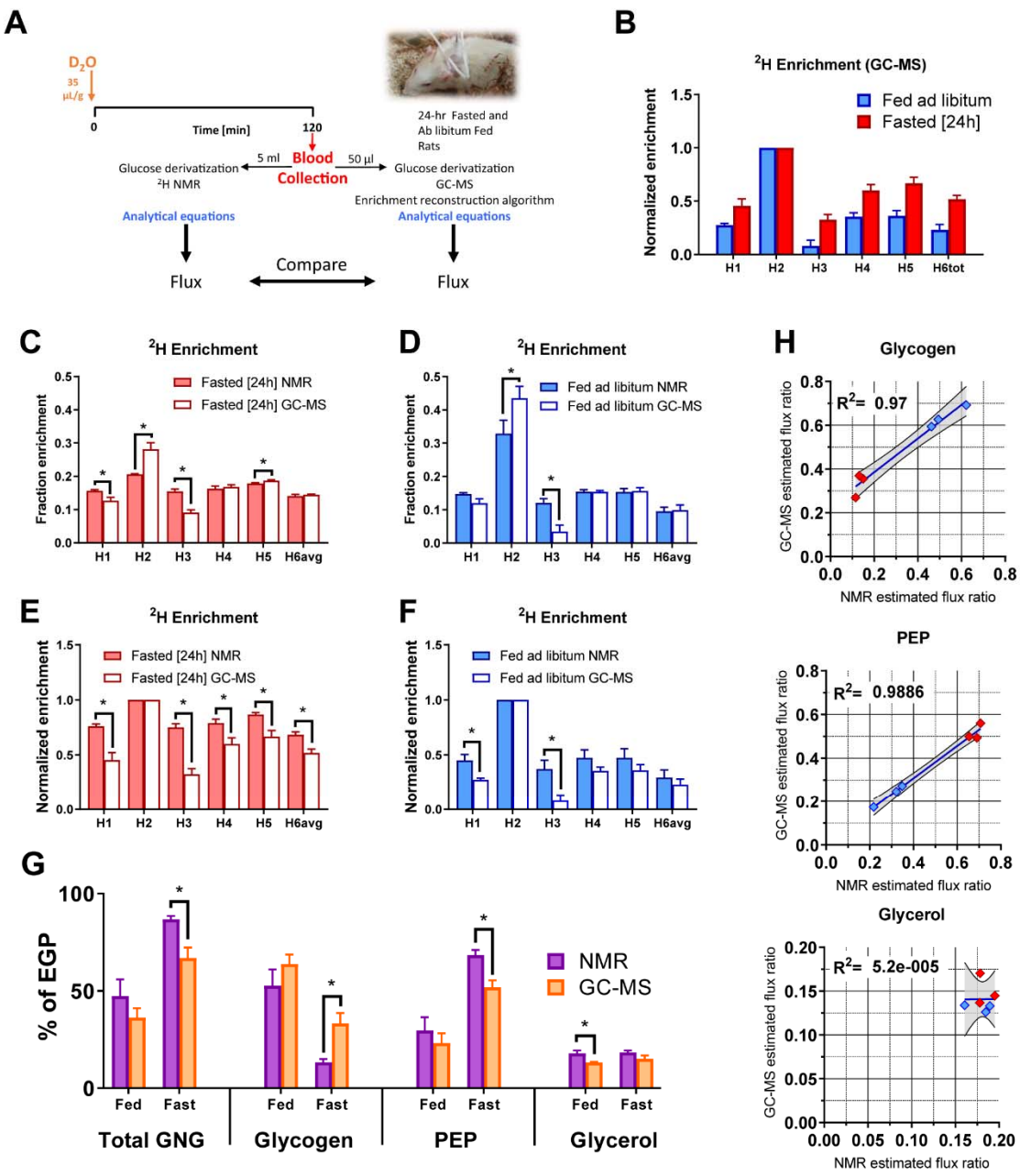
A
D2O
35 µL/g
0
Time [min]
120
24-hr Fasted and Ab libitum Fed Rats
Glucose derivatization
2H NMR
5 ml
Blood Collection
50 µl
Glucose derivatization
GC-MS
Enrichment reconstruction algorithm
Analytical equations
Analytical equations
Flux
Compare
Flux
B
2H Enrichment (GC-MS)
Fed ad libitum
Fasted [24h]
Normalized enrichment
H1
H2
H3
H4
H5
H6tot
C
2H Enrichment
Fasted [24h] NMR
Fasted [24h] GC-MS
Fraction enrichment
H1
H2
H3
H4
H5
H6avg
D
2H Enrichment
Fed ad libitum NMR
Fed ad libitum GC-MS
Fraction enrichment
H1
H2
H3
H4
H5
H6avg
H
Glycogen
R2= 0.97
GC-MS estimated flux ratio
NMR estimated flux ratio
E
2H Enrichment
Fasted [24h] NMR
Fasted [24h] GC-MS
Normalized enrichment
H1
H2
H3
H4
H5
H6avg
F
2H Enrichment
Fed ad libitum NMR
Fed ad libitum GC-MS
Normalized enrichment
H1
H2
H3
H4
H5
H6avg
PEP
R2= 0.9886
GC-MS estimated flux ratio
NMR estimated flux ratio
G
% of EGP
NMR
GC-MS
Fed
Fast
Total GNG
Glycogen
PEP
Glycerol
Glycerol
R2= 5.2e-005
GC-MS estimated flux ratio
NMR estimated flux ratio

Figure S1. Determination of glucose labeling form ^2H_2O by ^2H NMR and GC-MS. (**A**) Experimental setup of ^2H_2O glucose labeling *in vivo* in rats. After 120 min, blood was collected and divided into aliquots for parallel analysis of positional ^2H enrichment followed by calculation of metabolic fluxes using Landau's analytical equations (*Materials and Methods* Section 2.12), (**B**) Fractional enrichment of glucose obtained from fed *ad libitum* and 24h-fasted rats analyzed using GC-MS, (**C**) Fractional enrichment of glucose obtained from 24h fasted rats analyzed using ^2H NMR and GC-MS. (**D**) Fractional enrichment of glucose obtained from fed *ad libitum* rats analyzed using ^2H NMR and GC-MS. (**E**) Glucose labeling normalized to position H2 obtained from 24h fasted rats analyzed using ^2H NMR and GC-MS. (**F**) Glucose labeling normalized to position H2 obtained from fed *ad libitum* rats analyzed using ^2H NMR and GC-MS. (**G**) Differences in estimations of fluxes contributing to EGP between ^2H NMR and GC-MS. (**H**) Correlation between gluconeogenic flux ratios calculated using ^2H NMR and predicted ^2H labeling from GC-MS data. n=3 per group, *$p < 0.05$

Figure S2

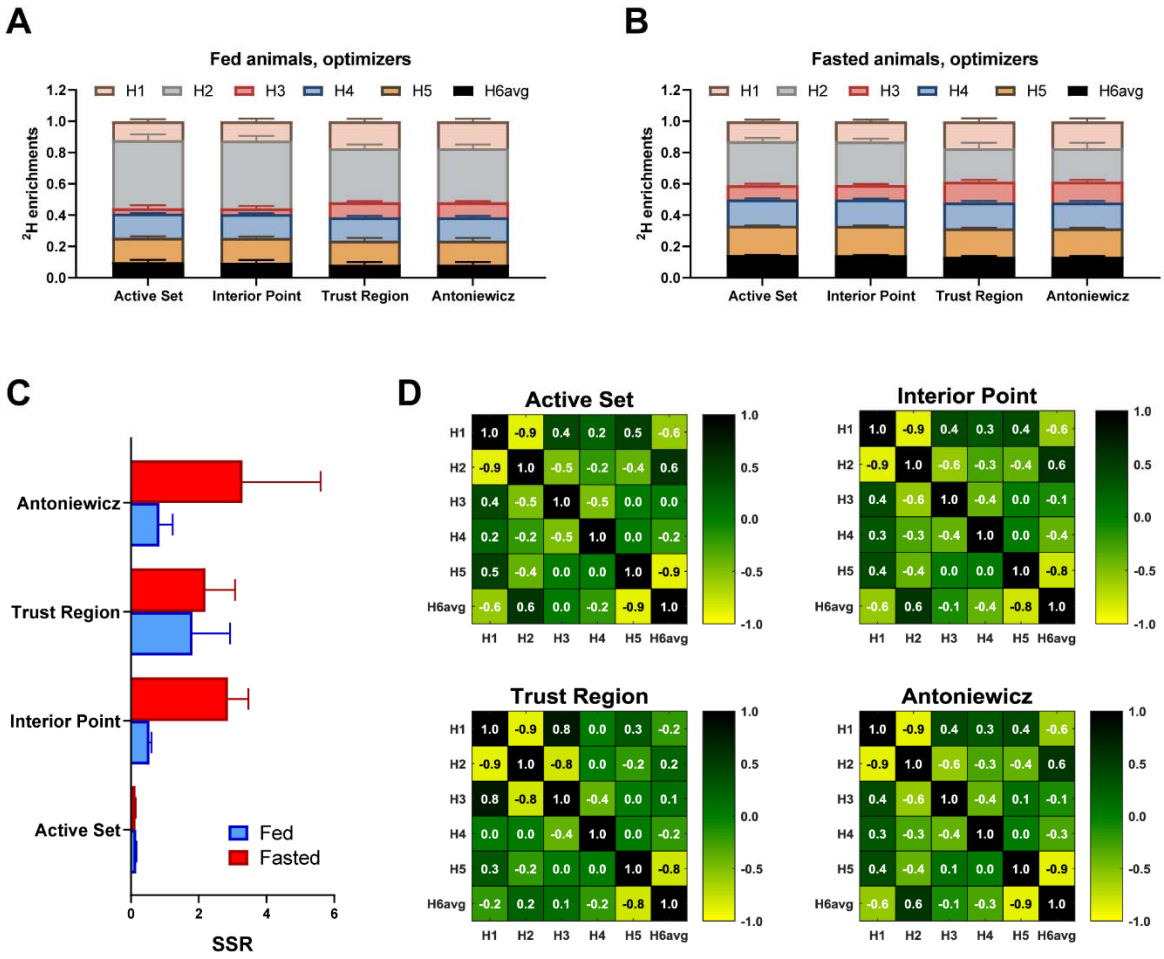
A
Fed animals, optimizers
H1 H2 H3 H4 H5 H6avg
^{2}H enrichments
Active Set
Interior Point
Trust Region
Antoniewicz
B
Fasted animals, optimizers
C
Antoniewicz
Trust Region
Interior Point
Active Set
Fed
Fasted
SSR
D
Active Set
Interior Point
Trust Region
Antoniewicz

Figure S2. Comparison of various optimizers used to estimate glucose ^{2}H enrichments from least-squares regression of GC-MS data. Three MATLAB quadratic programming solvers (active set, interior point, and trust region) were compared to the custom solver used by Antoniewicz et al. (2011). Unweighted least-squares regression was performed assuming 0.3 mol% error in the raw MID measurements. (**A**) Fractional ^{2}H enrichments of glucose obtained from fed *ad libitum* rats, (**B**) Fractional ^{2}H enrichments of glucose obtained from 24h-fasted rats, (**C**) Best-fit sum-of-squared residuals (SSRs) obtained from each solver, (**D**) Normalized parameter covariance matrices of estimated enrichments averaged over all samples. Error bars indicate SD (n=3).

Figure S3

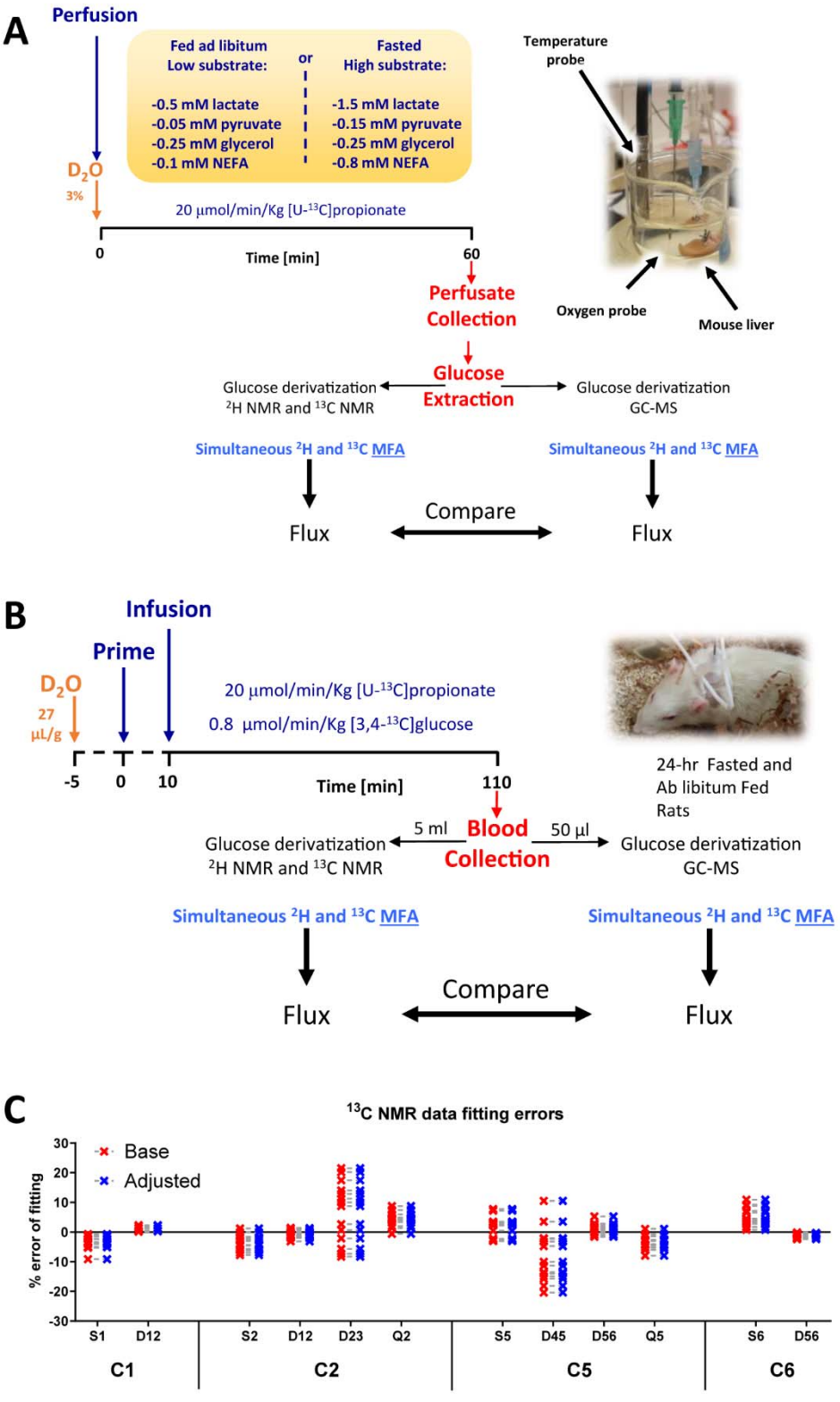
A
Perfusion
Fed ad libitum
Low substrate:
-0.5 mM lactate
-0.05 mM pyruvate
-0.25 mM glycerol
-0.1 mM NEFA
or
Fasted
High substrate:
-1.5 mM lactate
-0.15 mM pyruvate
-0.25 mM glycerol
-0.8 mM NEFA
D2O
3%
20 µmol/min/Kg [U-13C]propionate
0
Time [min]
60
Perfusate Collection
Glucose Extraction
Glucose derivatization
2H NMR and 13C NMR
Glucose derivatization
GC-MS
Simultaneous 2H and 13C MFA
Simultaneous 2H and 13C MFA
Flux
Compare
Flux
Temperature probe
Oxygen probe
Mouse liver
B
Infusion
Prime
D2O
27 µL/g
20 µmol/min/Kg [U-13C]propionate
0.8 µmol/min/Kg [3,4-13C]glucose
-5
0
10
Time [min]
110
Blood Collection
5 ml
50 µl
Glucose derivatization
2H NMR and 13C NMR
Glucose derivatization
GC-MS
24-hr Fasted and
Ab libitum Fed
Rats
Simultaneous 2H and 13C MFA
Simultaneous 2H and 13C MFA
Flux
Compare
Flux
C
13C NMR data fitting errors
Base
Adjusted
% error of fitting
30
20
10
0
-10
-20
-30
S1
D12
S2
D12
D23
Q2
S5
D45
D56
Q5
S6
D56
C1
C2
C5
C6

Figure S3. Double and triple tracer experiments. (**A**) Experimental setup of double tracer liver perfusion experiment (**B**) Experimental setup of triple tracer *in vivo* infusion in rats (**C**) Percent errors of fitting ^{13}C NMR data to base and adjusted model.

123 **Figure S4**

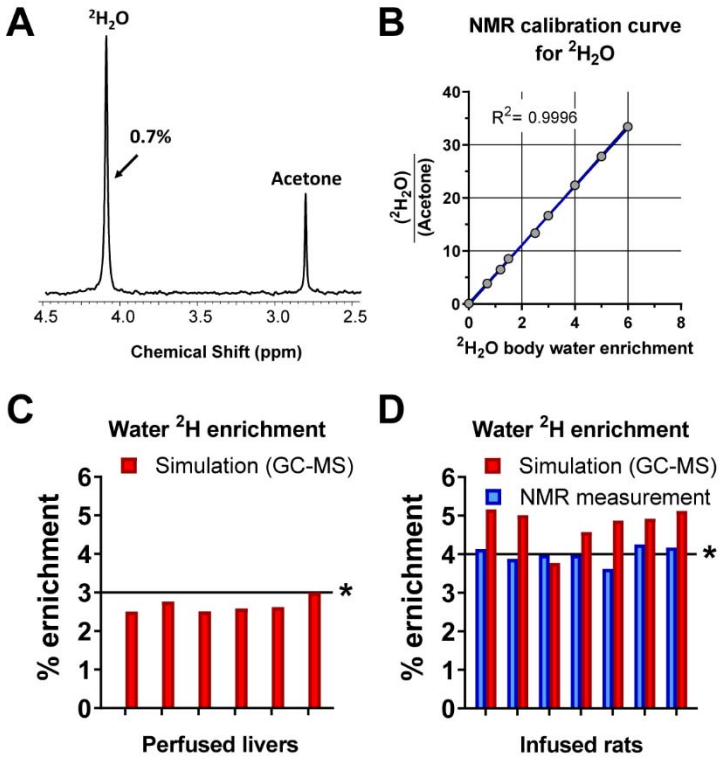

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Figure S4. Effect of body water enrichment. (**A**) ^{2}H NMR spectrum used for quantification of $^{2}H_2O$ enrichment in body water – note that acetone ^{2}H signal used as a reference originates from the natural deuterium abundance, (**B**) calibration curve for relationship between $^{2}H_2O$ enrichment in body water and the ratio of NMR signals ($^{2}H_2O$)/(Acetone), (**C**,**D**) $^{2}H_2O$ enrichment values based on simulation (from regression of GC-MS glucose data) and exact ^{2}H NMR measurements in (**C**) perfused livers and (**D**) tracer infusions in rats. Expected water ^{2}H enrichments are marked with horizontal line and asterisk (*).

Figure S5

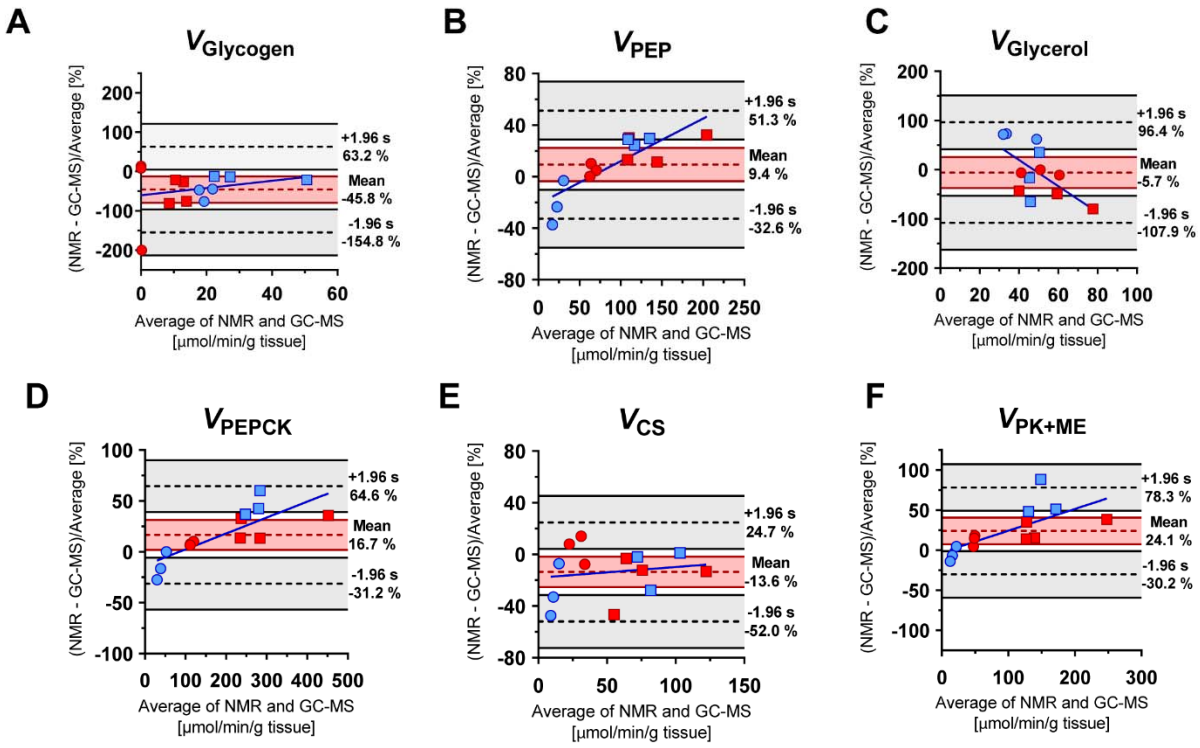
A
$V_{Glycogen}$
(NMR - GC-MS)/Average [%]
+1.96 s
63.2 %
Mean
-45.8 %
-1.96 s
-154.8 %
Average of NMR and GC-MS
[µmol/min/g tissue]
B
V_{PEP}
(NMR - GC-MS)/Average [%]
+1.96 s
51.3 %
Mean
9.4 %
-1.96 s
-32.6 %
Average of NMR and GC-MS
[µmol/min/g tissue]
C
$V_{Glycerol}$
(NMR - GC-MS)/Average [%]
+1.96 s
96.4 %
Mean
-5.7 %
-1.96 s
-107.9 %
Average of NMR and GC-MS
[µmol/min/g tissue]
D
V_{PEPCK}
(NMR - GC-MS)/Average [%]
+1.96 s
64.6 %
Mean
16.7 %
-1.96 s
-31.2 %
Average of NMR and GC-MS
[µmol/min/g tissue]
E
V_{CS}
(NMR - GC-MS)/Average [%]
+1.96 s
24.7 %
Mean
-13.6 %
-1.96 s
-52.0 %
Average of NMR and GC-MS
[µmol/min/g tissue]
F
V_{PK+ME}
(NMR - GC-MS)/Average [%]
+1.96 s
78.3 %
Mean
24.1 %
-1.96 s
-30.2 %
Average of NMR and GC-MS
[µmol/min/g tissue]

Figure S5. Bland-Altman analysis of agreement between NMR and GC-MS based flux estimates. Squares – tracer infusions in rats, circles – perfused mouse livers, blue – fed condition, red – fasted condition. All fluxes are reported in μmol/g liver/hr. (**A**) $V_{Glycogen}$, (**B**) V_{PEP}, (**C**) $V_{Glycerol}$, (**D**) V_{PEPCK}, (**E**) V_{CS}, (**F**) V_{PK+ME}.

138 **Figure S6**

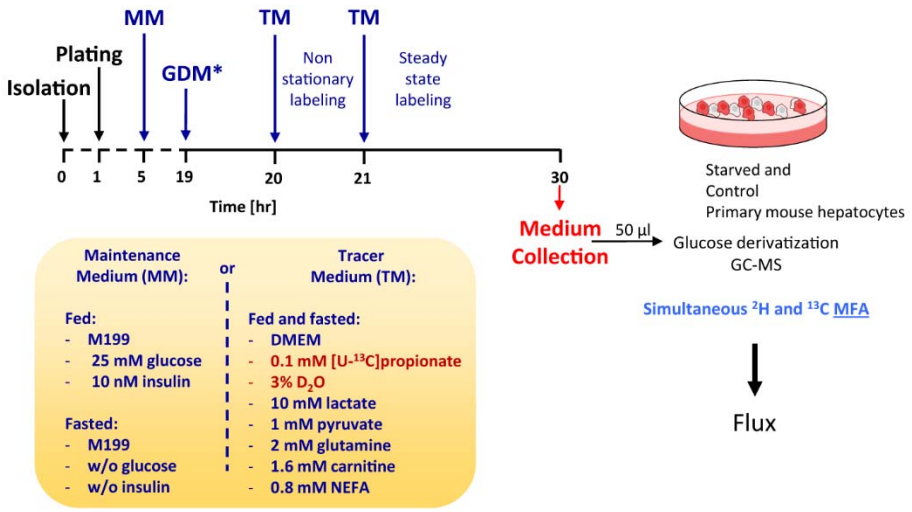

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Figure S6. Glucose labeling using double tracer method – *in vitro* primary mouse hepatocytes. Experimental setup of double tracer *in vitro* experiment. GDM – glycogen depletion media was additionally used in fasted condition.

Table S1. Metabolic network used for flux modeling.

Flux	Reaction	Base model	Adjusted model
V_{Inf}	Gluc.inf (AaBbCcDdEeFfg) -> Gluc.ext (AaBbCcDdEeFfg)	+	+
V_{EGP}	H6P (AaBbCcDdEeFfg) -> Gluc.ext (AaBbCcDdEeFfg)	+	+
$V_{Glycogen}$	Glycogen (AaBbCcDdEeFfg) + H (h) -> H6P (AaBhCcDdEeFfg) + H (b)	+	+
V_{GNG}	T3P (CcBhAab) + T3P (DdEeFfg) + H (i) -> H6P (AbBiCcDdEeFfg) + H (h) + H (a)	+	+
V_{GAPDH}	BPG (ABbCcd) + H (e) + H (f) -> T3P (AeBfCcd) + H (b)	+	+
$V_{Glycerol}$	Glycerol (AaeBbCcd) + H (f) -> T3P (AeBfCcd) + H (a) + H (b)	+	+
V_{PEP}	PEP (ABCcd) + H (b) -> BPG (ABbCcd)	+	+
V_{PK+ME}	PEP (ABCab) + H (c) -> Pyr (ABCabc)	+	+
V_{LDH}	Lac (ABbCcde) -> Pyr (ABCcde) + H (b)	+	+
V_{PC}	Pyr (ABCcde) + CO2 (D) + H (f) + H (g) -> 0.5*Oac (ABCfgD) + 0.5*Oac (DCBfgA) + H (c) + H (d) + H (e)	+	+
V_{PEPCK}	Oac (ABCabD) -> PEP (ABCab) + CO2 (D)	+	+
V_{FAT}	FAT (BCabc) -> AcCoA (BCabc)	+	+
V_{CS}	Oac (ABCcdD) + AcCoA (EFfgh) -> Cit (DCcdBFfgEA) + H (h)	+	+
V_{IDH}	Cit (ABabCDcdEF) + H (e) -> Akg (ABCeaDcdE) + H (b) + CO2 (F)	+	+
V_{OGDH}	Akg (ABCabDcdE) -> SucCoA (BCabDcdE) + CO2 (A)	+	+
V_{PCC}	PropCoA (ABabCcde) + CO2 (D) -> SucCoA (ACcdBabD) + H (e)	+	+
V_{SDH}	SucCoA (ABabCcdD) + H (e) + H (f) -> 0.5*Oac (ABCefD) + 0.5*Oac (DCBefA) + H (a) + H (b) + H (c) + H (d)	+	+
V_{Hinf}	H.inf (a) -> H (a)	+	+
V_{Hsink}	H -> Sink	+	+
$V^*_{Glyc\text{-}Aldo}$	Glycerol (AaeBbCcd) + H (f) + H (g) -> T3P (AgBfCcd) + H (a) + H (b) + H (e)	X	+
$V^*_{TPI\text{-}KIE}$	H6P (AaBbCcDdEeFfg) + H.src (h) -> H6P (AaBbChDdEeFfg) + H (c)	X	+
V^*_{PMI}	H6P (AaBbCcDdEeFfg) + H (h) -> H6P (AhBbCcDdEeFfg) + H (a)	X	+
V^*_{TAL}	H6P (AaBbCcDdEeFfg) + T3P (HhJjKkm) + H (n) -> H6P (AaBbCcHhJjKkm) + T3P (DdEnFfg) + H (e)	X	X

Atom transitions are denoted by letters: capital for carbon atoms and lower case for protons. Additional tested assumptions are labeled with an asterisk (*). Note that V_{Inf} was only used for triple tracer experiments where [3,4-$^{13}C_2$]glucose was infused *in vivo*. Green + denotes reactions that were used in the model.

Table S2. Accuracy and precision of MIDs measured from mouse blood

	Fragment (formula)		M+0	M+1	M+2	M+3	M+4
DIO	m/z 301	exp	84.4 ± 0.03	13.2 ± 0.01	2.2 ± 0.01	0.2 ± 0.00	0.0 ± 0.01
	(C14H21O7)	theory	84.2	13.4	2.2	0.2	0.0
MOX	m/z 145	exp	92.1 ± 0.07	6.7 ± 0.01	1.0 ± 0.02	0.1 ± 0.00	0.1 ± 0.00
	(C6H11O3N)	theory	92.5	6.7	0.8	0.0	0.0
ALDO	m/z 173	exp	90.4 ± 0.02	8.0 ± 0.01	1.1 ± 0.01	0.1 ± 0.00	0.0 ± 0.00
	(C8H13O4)	theory	90.6	8.3	1.1	0.1	0.0
	m/z 259	exp	86.5 ± 0.01	11.5 ± 0.02	1.8 ± 0.02	0.2 ± 0.01	0.0 ± 0.01
	(C12H19O6)	theory	86.2	11.8	1.8	0.2	0.0
	m/z 284	exp	84.8 ± 0.05	12.7 ± 0.05	2.0 ± 0.01	0.2 ± 0.02	0.1 ± 0.02
	(C13H18O6N)	theory	85.0	12.8	1.9	0.2	0.0
	m/z 370	exp	81.3 ± 0.09	15.5 ± 0.07	2.9 ± 0.01	0.4 ± 0.01	0.0 ± 0.01
	(C17H24O8N)	theory	80.9	15.9	2.8	0.4	0.0

Experimental (exp) and theoretical (theory) MID abundances of three investigated glucose derivatives. Molar percent abundances (mol %) are reported as mean ± SD, n = 4. Maximum deviation was 0.4% and measured precision was below 0.1%. Note that this result satisfies the accuracy criteria (accuracy at least 0.5%, precision at least 0.1%) used for selection of these six glucose m/z fragments in original work by Antoniewicz (2011).

Table S3. Comparison of GC-MS based estimation of glucose ^{2}H enrichment with exact NMR enrichments

			Fed				Fasted				p value[a]
			Rat1	Rat2	Rat6	Mean ± SD	Rat3	Rat4	Rat5	Mean ± SD	
Glucose fractional ^{2}H enrichment %	H1	NMR	15.0	14.3	14.9	14.7 ± 0.4	15.5	15.5	16.0	15.7 ± 0.3	0.03
		GC-MS	11.5	11.1	13.4	12.0 ± 1.2	12.7	11.7	13.7	12.7 ± 1.0	0.52
		p value[b]				0.05				0.03	
	H2	NMR	29.8	31.4	37.4	32.9 ± 4.0	20.7	20.6	20.4	20.6 ± 0.2	0.03
		GC-MS	40.4	42.9	47.4	43.5 ± 3.6	28.8	29.7	25.9	28.1 ± 2.0	0.01
		p value[b]				0.03				0.02	
	H3	NMR	12.8	12.9	10.5	12.1 ± 1.3	16.2	14.7	15.5	15.5 ± 0.7	0.03
		GC-MS	5.3	3.7	1.4	3.5 ± 2.0	9.4	8.1	9.7	9.1 ± 0.8	0.02
		p value[b]				0.00				0.00	
	H4	NMR	16.0	15.5	14.9	15.4 ± 0.6	16.3	17.0	15.4	16.3 ± 0.8	0.23
		GC-MS	15.5	15.7	15.0	15.4 ± 0.4	16.1	17.2	17.2	16.8 ± 0.6	0.04
		p value[b]				0.90				0.41	
	H5	NMR	16.0	15.9	14.2	15.4 ± 1.0	17.6	17.9	18.1	17.9 ± 0.2	0.05
		GC-MS	16.4	16.0	14.6	15.7 ± 0.9	18.6	18.7	18.9	18.7 ± 0.2	0.03
		p value[b]				0.74				0.01	
	H6avg	NMR	10.4	10.1	8.1	9.5 ± 1.2	13.6	14.2	14.5	14.1 ± 0.4	0.01
		GC-MS	11.0	10.6	8.2	9.9 ± 1.5	14.4	14.6	14.5	14.5 ± 0.1	0.03
		p value[b]				0.74				0.23	
% EGP	Glycogen	NMR	46.2	49.4	62.2	52.6 ± 8.4	15.0	13.2	11.5	13.2 ± 1.7	0.01
		GC-MS	59.5	62.7	69.2	63.8 ± 5.0	35.6	37.0	26.9	33.2 ± 5.4	0.00
		p value[b]				0.14				0.02	
	Glycerol	NMR	18.9	18.5	16.1	17.8 ± 1.5	19.5	17.8	17.8	18.4 ± 1.0	0.64
		GC-MS	13.3	12.6	13.4	13.1 ±0.4	14.5	13.7	17.0	15.1 ± 1.8	0.19
		p value[b]				0.03				0.06	
	PEP	NMR	34.9	32.1	21.8	29.6 ± 6.9	65.5	69.0	70.7	68.4 ± 2.6	0.01
		GC-MS	27.2	24.7	17.4	23.1 ± 5.1	50.0	49.4	56.0	51.8 ± 3.7	0.00
		p value[b]				0.27				0.00	

[a] Student's t test comparing fed and fasted conditions

[b] Student's t test comparing NMR and GC-MS data for individual positions

Table S4. Analysis of agreement between methods

Liner regression statistics		$V_{Glycogen}$	V_{PEP}	V_{PEPCK}	V_{PK+ME}	V_{CS}	$V_{Glycerol}$
Goodness of Fit	P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.7552
	r^2	0.9215	0.9741	0.9305	0.8649	0.945	0.009207
	r	0.9599	0.9870	0.9646	0.9300	0.9721	0.0960
	Slope	1.17	0.6842	0.6204	0.5691	1.08	-0.2332
	Intercept	3.421	14.3	28.55	13.85	2.259	63.76
Passing Bablok regression statistics							
Slope	Value	1.2329	0.6757	0.6358	0.6396	1.0725	N/A
	CI [LB]	1.0707	0.622	0.5274	0.4915	0.9327	N/A
	CI [UB]	1.9757	0.818	0.821	0.7799	1.2786	N/A
	Proportional differences? [a]	**Yes**	**Yes**	**Yes**	**Yes**	**No**	N/A
Intercept	Value	0.4006	11.8454	22.6028	9.0768	0.215	N/A
	CI [LB]	-4.9571	6.4599	10.2231	3.8916	-8.3192	N/A
	CI [UB]	2.5748	18.9534	47.1028	19.4755	5.8618	N/A
	Constant differences? [b]	**No**	**Yes**	**Yes**	**Yes**	**No**	N/A
Bland Altman bias analysis							
Bias [%]	Value	-45.8	9.4	16.7	24.1	-13.6	-5.7
	CI [LB]	-79.4	-3.6	1.9	7.3	-25.4	-37.2
	CI [UB]	-12.2	22.3	31.4	40.8	-1.8	25.7

CI – confidence interval, LB – lower boundary, UB – upper boundary

[a] Does the slope CI enclose the value of 1? If no, there is evidence for proportional difference between analyzed methods.

[b] Does the intercept CI enclose the value of 0? If no, there is evidence for constant differences (bias) between analyzed methods.