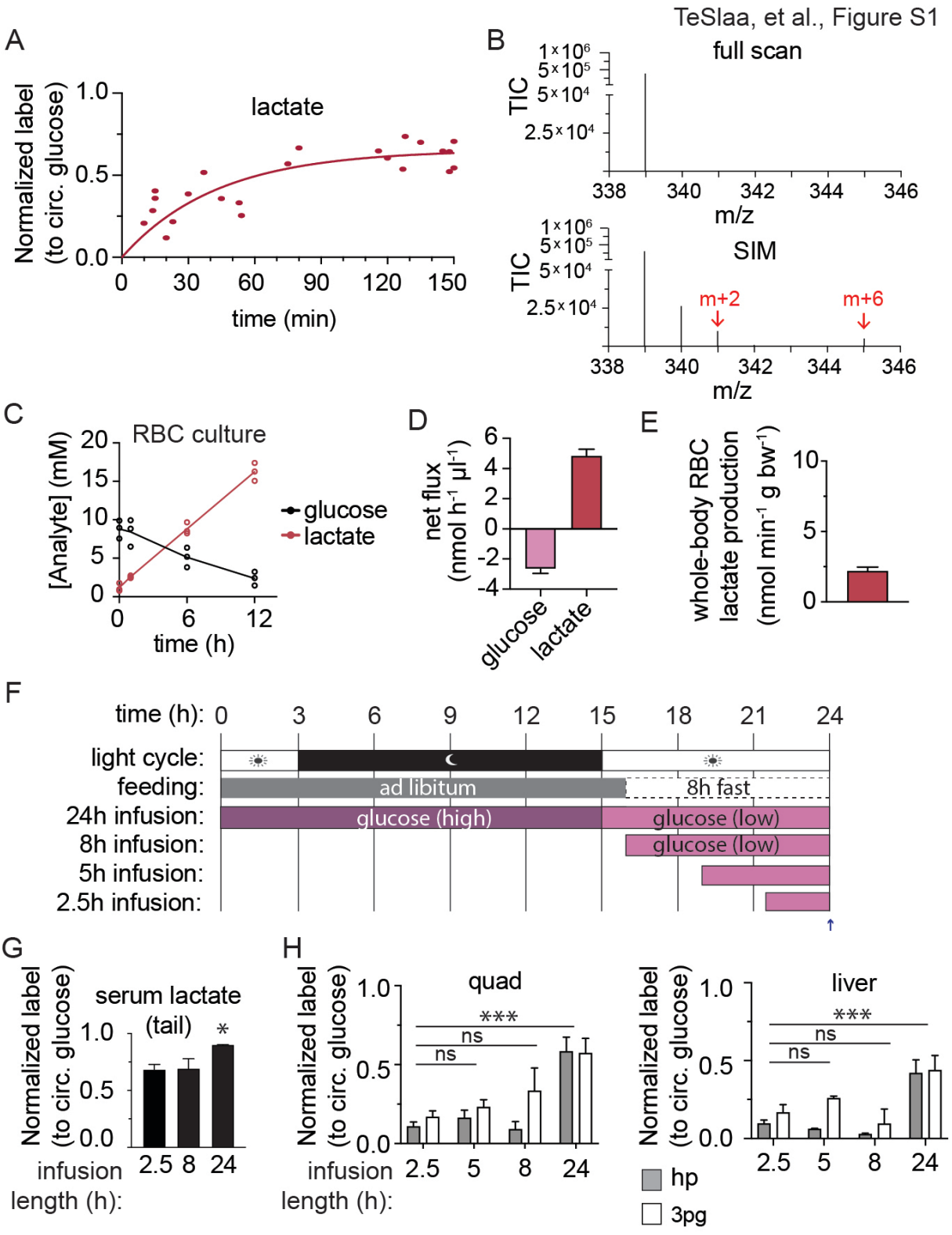

Figure S1. Optimization of glucose infusions to measure the contribution of glucose to glycolytic intermediates. Related to Figure 1.

(A) Labeling of circulating lactate normalized to circulating glucose labeling during 2.5 h infusion of [U-^{13}C]glucose. Blue arrow indicates time of serum and tissue collection.

(B) Mass spectra fructose-1,6-bisphophate and its labeled forms detected by either full scan of m/z 80-1000 or SIM scan of m/z from 336-350 by LC-MS. Both spectra are from quadricep muscle extract collected from 2.5 h [U^{13}C]glucose infusion with circulating glucose labeling of 19%.

(C-E) Data from *ex vivo* red blood cells (RBC) culture. (C) Concentration of glucose and lactate during culture of freshly isolated mouse whole blood. (n=3 mice). (D) Rates of glucose and lactate production normalized to packed red blood cell volume. (n=10). (E) Rate of whole-body lactate production from RBCs calculated assuming murine blood volume of 0.07 ml per g body weight and RBC volume 40% of total blood volume and fluxes measured in panel (D).

(F) Schematic showing [U-^{13}C]glucose infusions of various lengths in comparison to the 2.5 h infusion.

(G) Labeling of serum lactate collected from the tail after 2.5 h (n=6 mice), 8 h (n=2), or 24 h (n=4) of [U-^{13}C]glucose infusion. P-value from one-way ANOVA; *p=0.016

(H) Labeling of quadriceps and liver glycolytic intermediates hexose phosphate (hp) and 3-phosphoglycerate (3pg) from after 2.5 h (n=5 mice), 5 h (n=2), 8 h (n=2), and 24 h (n=4). P-value is from two-way ANOVA with tukey correction for multiple comparisons; ***p<0.001.
Mean ± SEM

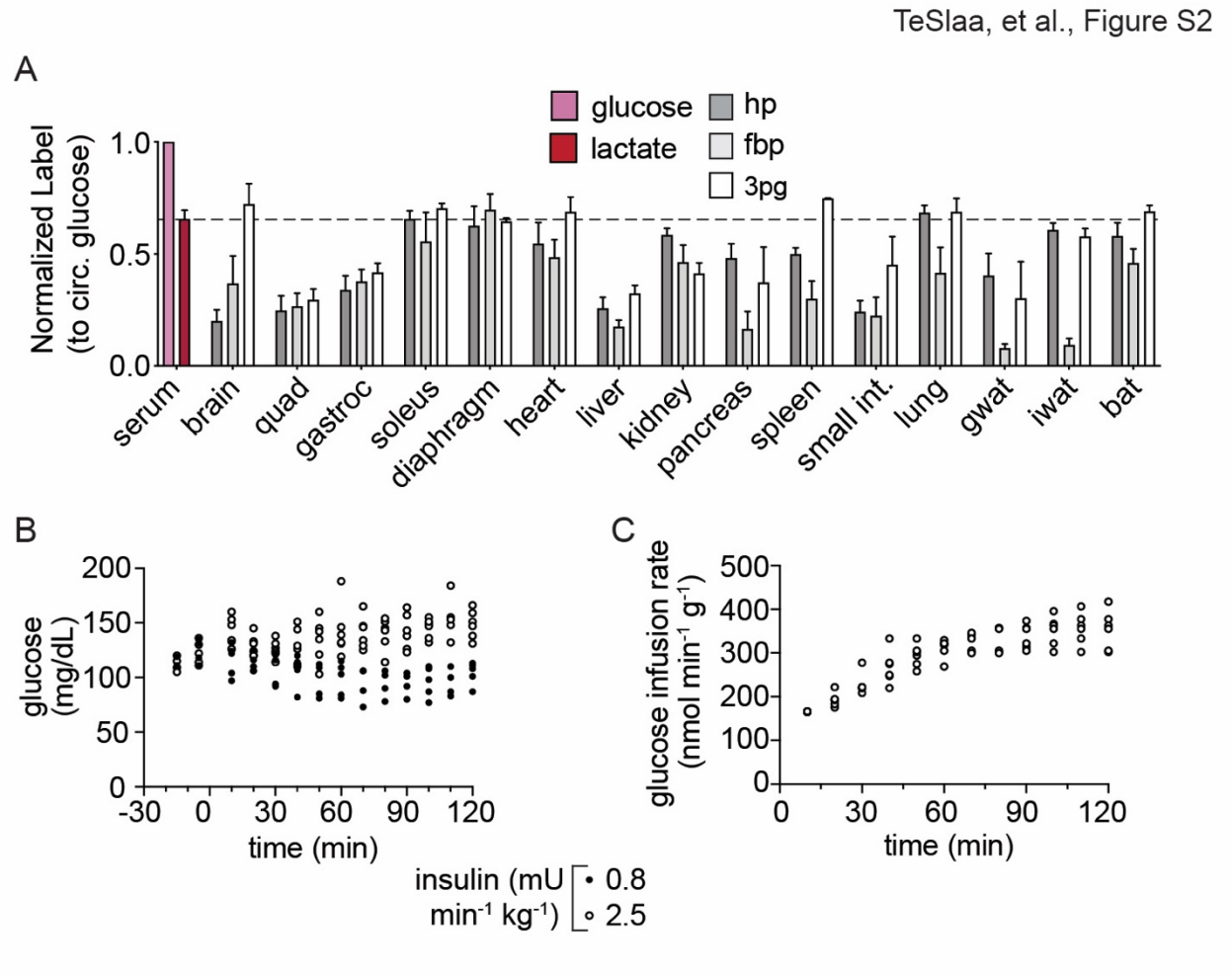

Figure S2. Impact of feeding and insulin on glucose use in glycolysis. Related to Figure 2 and 7.

(A) Labeling in arterial serum glucose, arterial serum lactate (n=6 from 1D), and tissue glycolytic intermediates at the end of 2.5 h refed [U-^{13}C]glucose infusion. n=3 for most tissues except small intestine, bat, brain (n=5) and quad, heart, liver, and kidney (n=6). Tissue abbreviations are quadriceps fermoris muscle (quad), gastrocnemius muscle (gastroc), small intestine (small int), gonadal white adipose tissue (gwat), inguinal white adipose tissue (iwat), and brown adipose tissue (bat).

(B) Blood glucose concentration during insulin infusion of 0.8 or 2.5 mU min^{-1} kg^{-1} from arterial catheter sampling.

(C) Glucose infusion rate to maintain euglycemia during 2.5 mU min^{-1} kg^{-1} infusion of insulin.

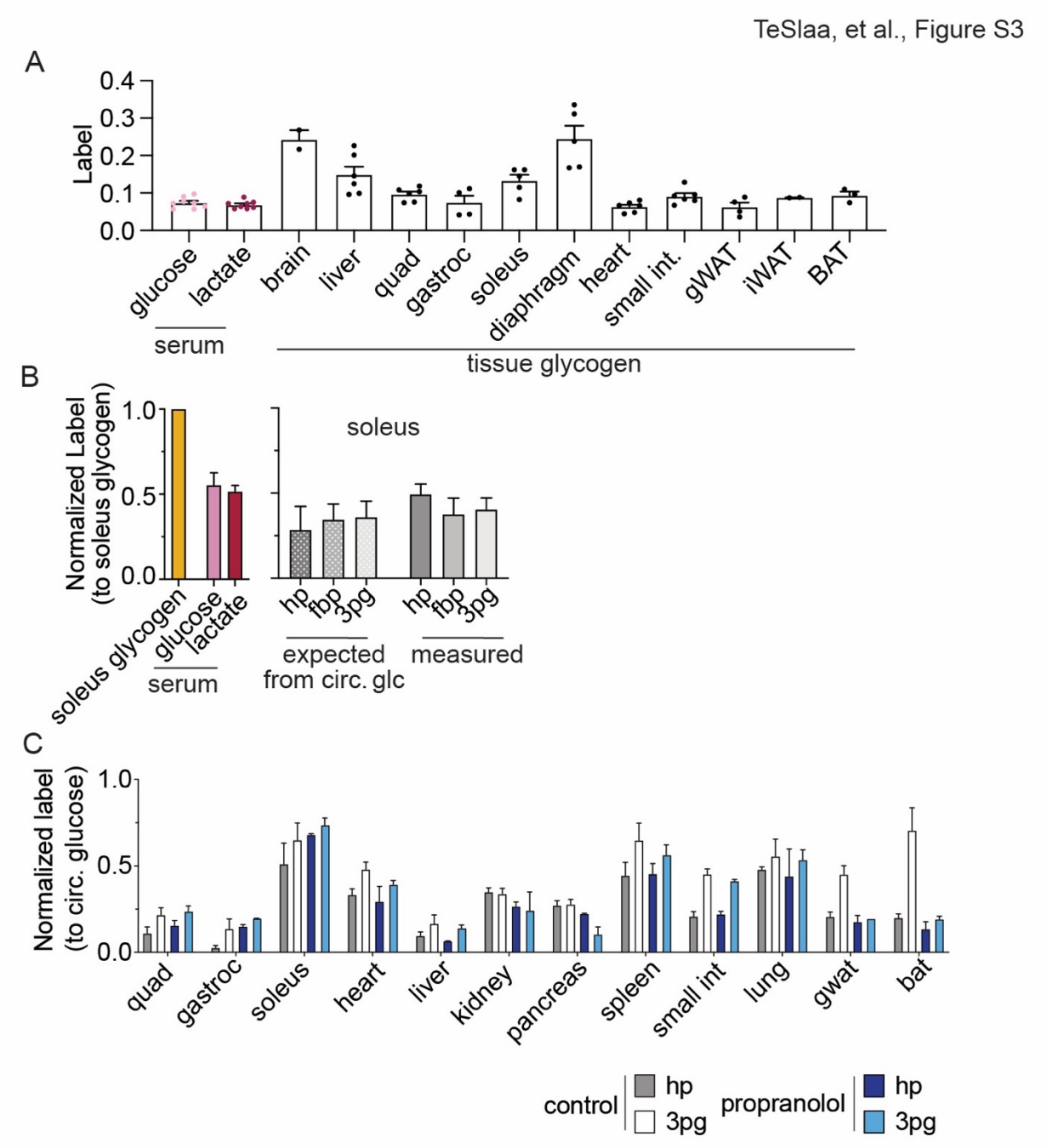

Figure S3. Glycogen supplies glycolytic intermediates in all tissues. Related to Figure 3.

(A) Labeling of serum glucose, serum lactate and tissue glycogen at the end point of the fasted pulse-chase infusionb. n vary by tissue; n=4 mice for most tissues; n=6 for liver, quad, heart, and small intestine; n=5 for soleus and diaphragm, n=3 for iwat, bat and gastroc, and n=2 for brain.

(B) Labeling in serum glucose and lactate and tissue glycolytic intermediates normalized to soleus glycogen at the end of the pulse-chase experiment, analogous to main text Figure 3C,D for liver and quadriceps; n=5 mice.

(C) Labeling of glycolytic intermediates normalized to labeling in circulating glucose after 2.5 h infusion of [U-^{13}C]glucose with or without treatment with 50 mg/kg propranolol. Data without propranolol is repeated from Figure 1E; n=2 mice for propranolol treatment and n=6 for control except in gastroc (n=2), soleus (n=4), and bat (n=4).
Mean ± SEM.

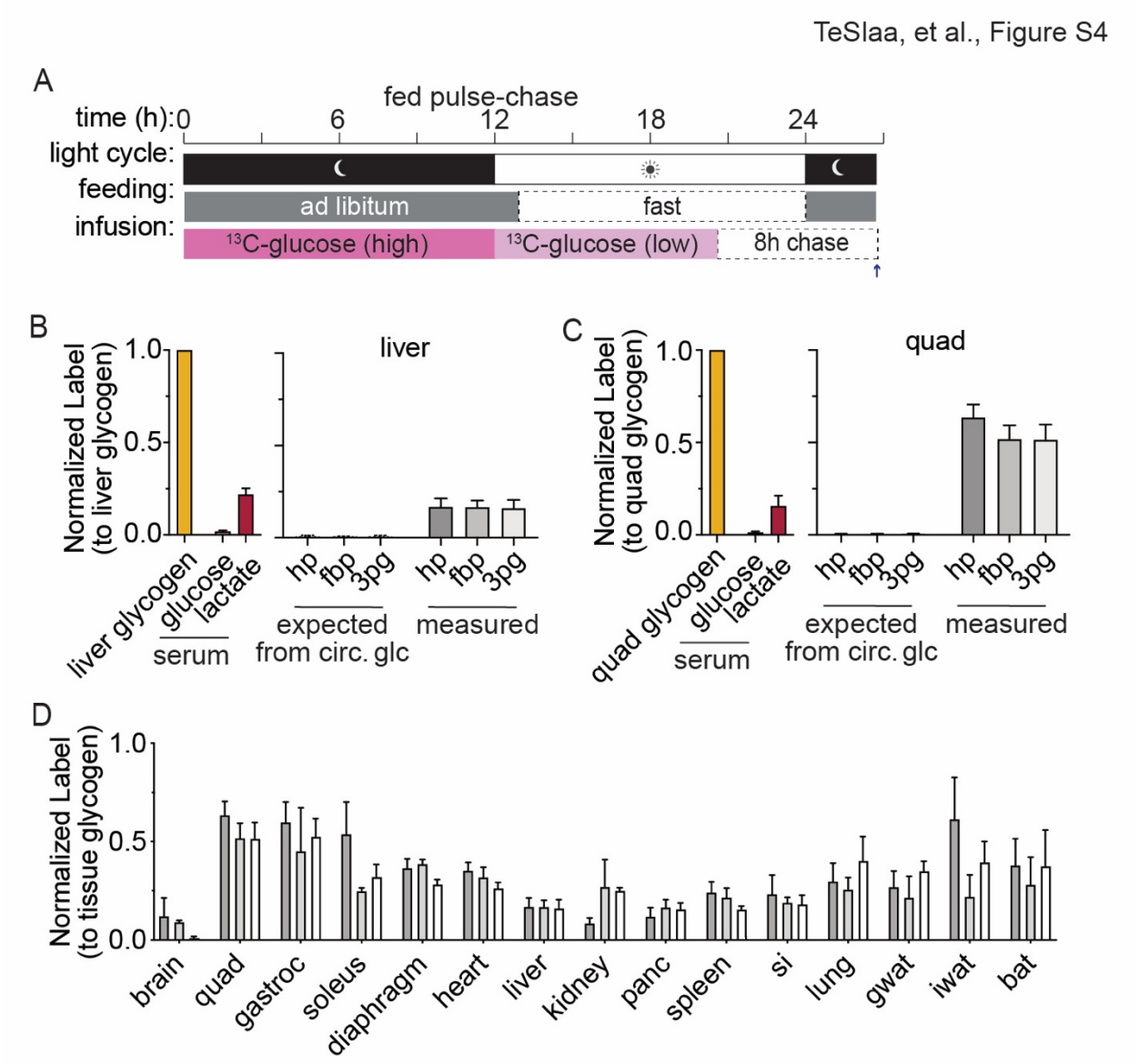

Figure S4. Pulse chase infusion in the fed state. Related to Figure 3 and 7.

(A) Schematic of fed pulse-chase experiment. Mice were infused with [U-^{13}C]glucose for 19 h with the goal of labeling glycogen. Next an 8 h chase was performed in which the infusion was stopped during 8 h of fasting to allow circulating metabolite labeling to decrease. Blue arrow indicates timing of final serum collection and tissue collection.

(B-C) Fed state experiment analogous to the fasted state data in main text Figure 3C,D. Labeling in serum glucose and lactate (n=6 mice) and tissue glycolytic intermediates normalized to glycogen labeling in the liver (n=5 mice) (B) or the quadriceps (n=4 mice) (C) at the end of the fed pulse-chase experiment. Expected labeling from circulating glucose is calculated by the fed 2.5 h glucose infusion data from Figure 2 as described by the displayed equation.

(D) Labeling in glycolytic intermediates at the end of the fed pulse chase experiment normalized to glycogen labeling measured in each tissue. n vary by tissue; n=6 mice for pancreas and spleen; n=5 for liver, kidney, and bat; n=4 for quad, heart, lung, and gastroc; n=3 for iwat and soleus, and n=2 for diaphragm, gwat and brain.
Mean ± SEM.

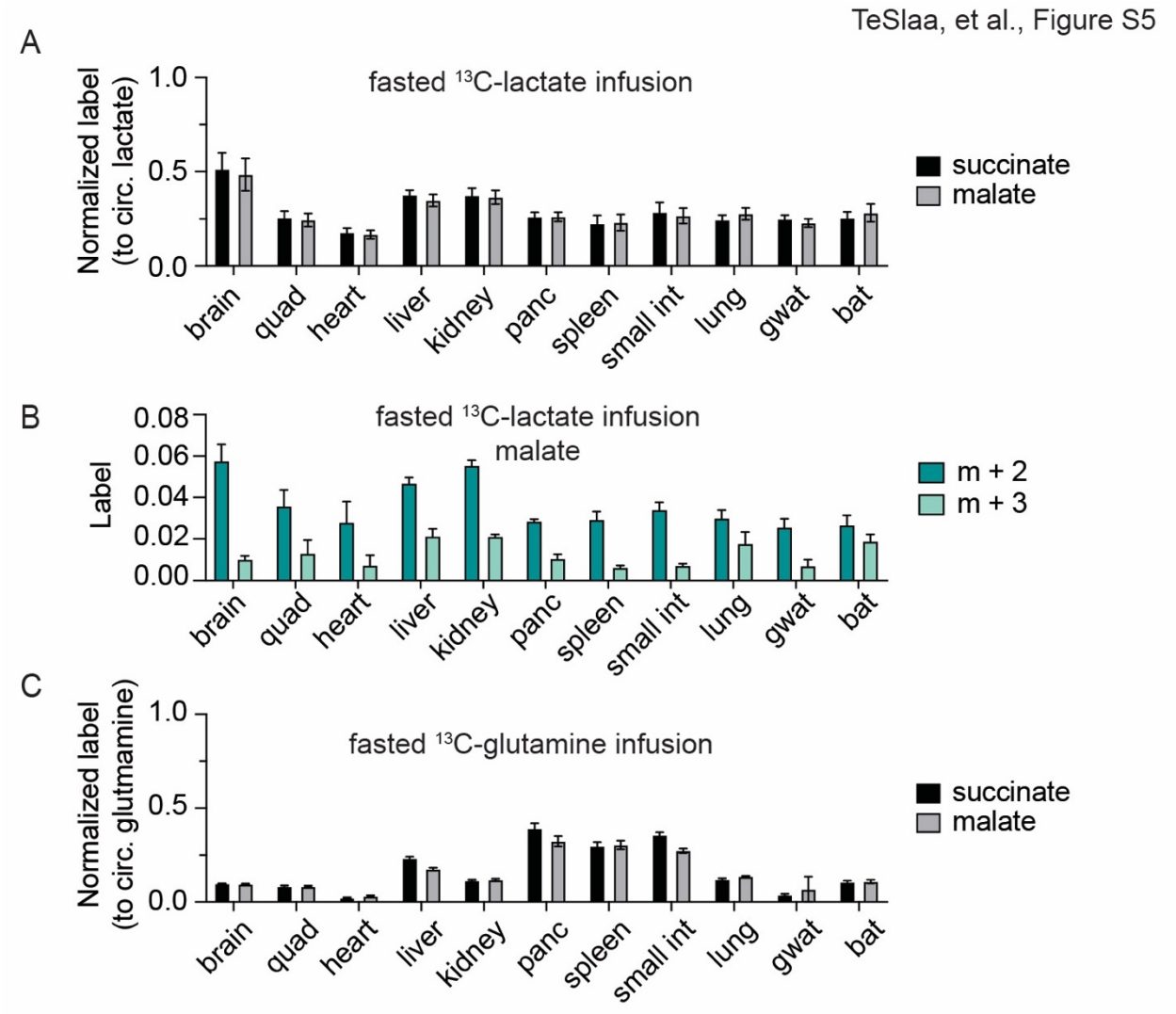

Figure S5. Labeling of TCA metabolites from lactate and glutamine. Related to Figure 4.

(A-C) Labeling in tissue TCA metabolites succinate and malate after 2.5 h fasted infusion (normalized to enrichment of the tracer in serum). (A) and (B) are [U-^{13}C]lactate infusion. Panel (A) shows that malate and succinate data agree. L (B) shows the relative abundance of M+2 and M+3 labeled forms (from TCA turning and anaplerosis, respectively). (C) is [U-^{13}C]glutamine infusion.
Mean ± SEM.

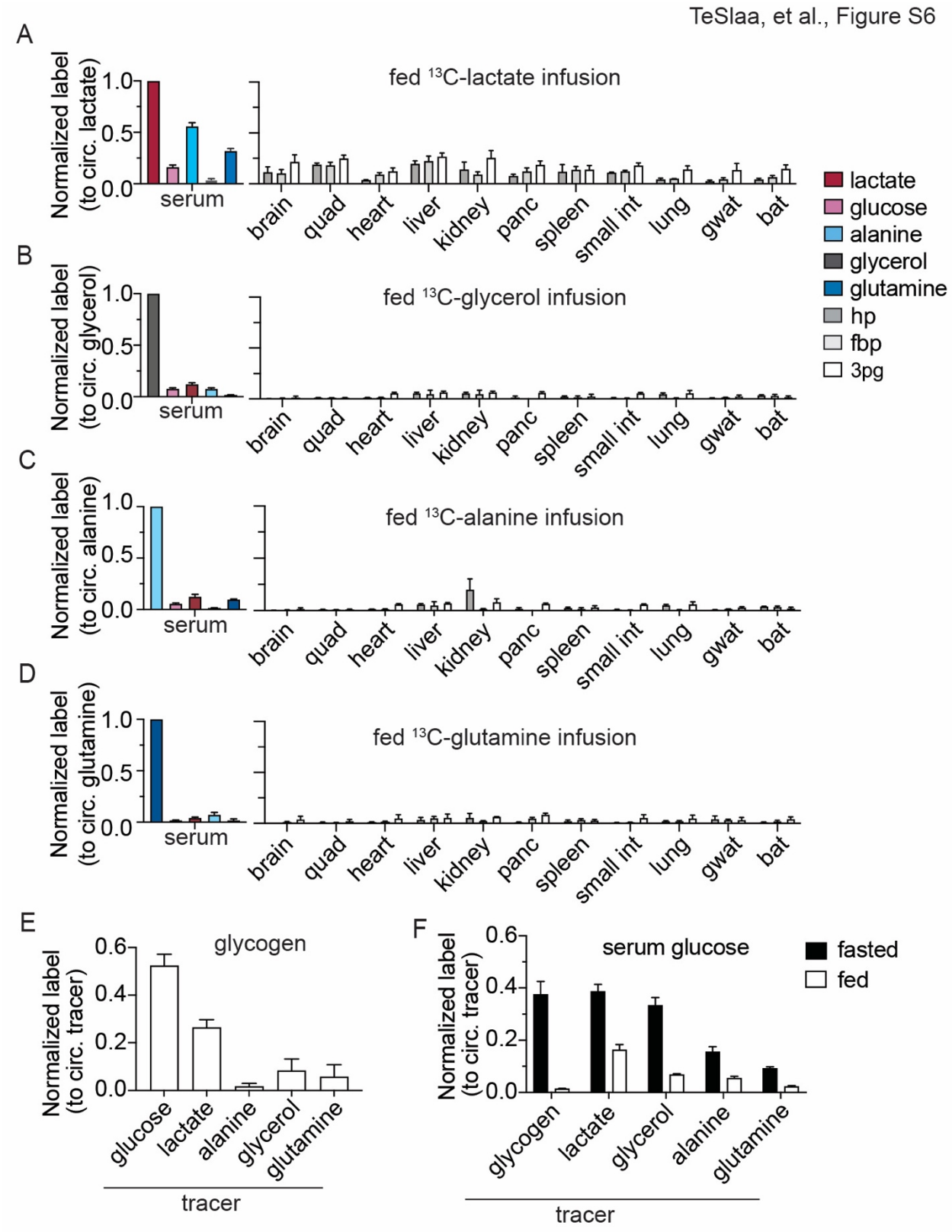

Figure S6. Lactate contributes to glycolytic intermediates in the fed state. Related to Figures 4, 5, and 7.

(A-D) Labeling in circulating metabolites (lactate, glucose, alanine, glycerol, and glutamine) and tissue glycolytic intermediates after 2.5 h fed infusion (normalized to enrichment of the tracer in serum). Each panel represents data from a different tracer: [U-^{13}C]lactate (A), [U-^{13}C]glycerol (B), [U-^{13}C]alanine (C), and [U-^{13}C]glutamine (D). n=4 mice in all cases except for in [U-^{13}C]lactate infusion where n=7 for serum and liver and kidney measurements (n=8 mice).

(E) Labeling of glycogen from 2.5 h fed [U-^{13}C] glucose (n=2), lactate (n=3), glycerol (n=2), alanine (n=3), or glutamine (n=3) infusion.

(F) Labeling of serum glucose during fasted pulse chase (n=6), fasted lactate (n=10), fasted glycerol (n=4), fasted alanine (n=5), fasted glutamine (n=4), fed pulse chase (n=3), fed lactate (n=7), fed glycerol (n=3), fed alanine (n=3), and fed glutamine (n=3) infusion experiments.

Mean ± SEM.

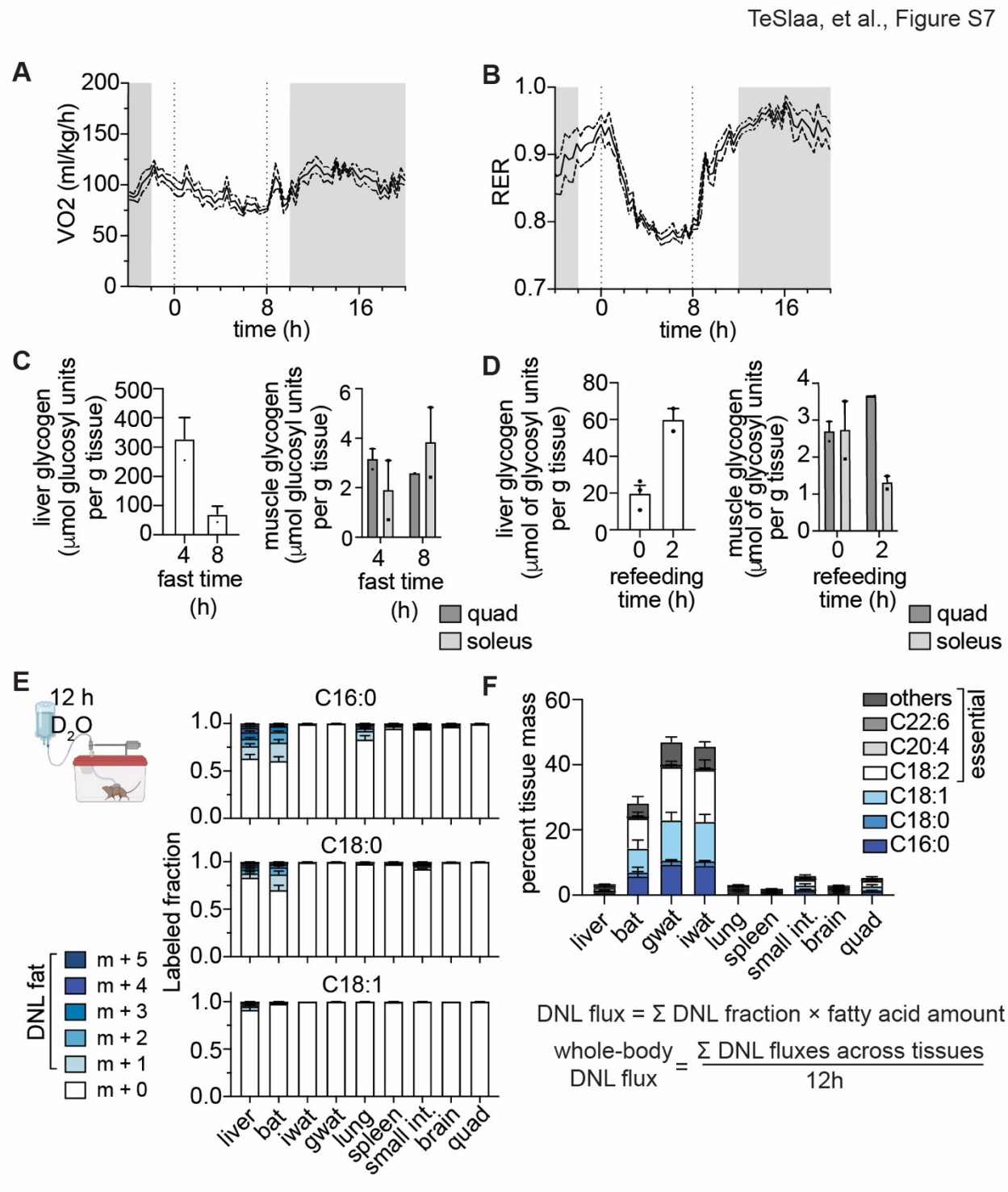

Figure S7. Quantification of glucose consuming pathways.
Related to Figure 6.

(A-B) Oxygen consumption (VO2) (A) and respiratory exchange ratios (RER) (B) from previously reported indirect calorimetry (Hui et al., 2020); n=6 per condition.

(C-D) Concentration of glycogen measured in liver and muscle after fasted state (C) or fed state (D) experiments (n=2).

(E) Labeling of saponified fatty acids from 12 h D_2O infusion (n=3).

(F) Fractional contribution of each fatty acid to tissue weight.
Mean ± SEM.

		Concentration (μM)				
		glycolytic intermediates			TCA intermediates	
		hexose-phosphate	fructose-1,6-bis-phosphate	3-phospho glycerate	succinate	malate
TISSUES	quad	4334.8 ± 547.9	231.6 ± 11.1	402.1 ± 11.8	2218.1 ± 30.3	705.4 ± 39.2
	gastroc	301.8 ± 303.3	186.4 ± 32.1	296.0 ± 59.1	2085.3 ± 249.5	560.9 ± 145.4
	soleus	881.9 ± 46.0	445.8 ± 178.5	434.5 ± 6.9	2773.5 ± 133.2	1663.9 ± 98.0
	heart	349.3 ± 144.2	579.4 ± 318.5	242.2 ± 81.8	2902.2 ± 72.4	1119.0 ± 162.8
	liver	391.3 ± 37	63.2 ± 2.5	150.9 ± 18.6	1491.7 ± 134.7	1586.0 ± 106.0
	kidney	74.7 ± 2.6	99.1 ± 6.1	90.5 ± 10.8	2249.0 ± 72.4	1679.2 ± 40.0
	pancreas	47.2 ± 2.8	63 ± 1.6	58.6 ± 10.8	1209.5 ± 41.8	1182.5 ± 91.0
	spleen	46.6 ± 2.3	108.6 ± 7.8	107.9 ± 6.6	630.0 ± 40.3	1154.3 ± 94.8
	small intestine	101.4 ± 24.0	109.5 ± 2.5	112.1 ± 25.2	1600.0 ± 319.2	1767.6 ± 600.5
	lung	137.6 ± 24.4	192 ± 20.6	96.2 ± 12.9	480.2 ± 185.9	1219.1 ± 96.1
	gwat	26.7 ± 0.2	54.4 ± 1.3	8.9 ± 0.3	50.2 ± 4.2	127.8 ± 4.3
	iwat	47.9 ± 13.9	60.8 ± 1.2	13.5 ± 0.9	104.3 ± 15.8	155.3 ± 29.1
	bat	117.4 ± 29.3	72.8 ± 11.4	22.9 ± 0.9	1897.8 ± 261.3	810.3 ± 343.2
CELLS	A549	33.6 ± 1.2	160.7 ± 25.2	93.0 ± 7.5	226.4 ± 7.2	2692.2 ± 227.7
	HCT116	69.5 ± 3.7	151.7 ± 15	56.5 ± 4	422.7 ± 11.7	2185.8 ± 120.2
	MIA PaCa-2	55.3 ± 1.4	555 ± 66.4	217.9 ± 21.2	833.0 ± 4.0	5119.6 ± 162.2
	Panc1	93.7 ± 0.4	851.6 ± 70.5	146.5 ± 2.7	626.2 ± 13.9	3778.8 ± 169.7

Table S1. Concentration of central carbon metabolites in mouse tissues and cell lines in culture. Related to Figure 7. Concentrations of indicated metabolites measured by LC-MS in tissues of ad libitum fed C57Bl/6 mice during the dark cycle

(11pm) and cell lines cultured in medium containing 1g/L glucose. Abbreviations: quadricep muscle (quad), gastrocnemius muscle (gastroc), gonal white adipose tissue (gwat), inguinal white adipose tissue (iwat), brown adipose tissue (bat). Mean ± SEM; n=2 biological replicates.