Supplemental Method 1: Carbohydrate fluxes – rates of interconversion and oxidation.

Using data from the various infusions in this paper (2.5 h glucose infusion, pulse-chase glucose infusion, lactate infusion, glycerol infusion, alanine infusion, and glutamine infusion), we can solve for the influxes to each of these circulating metabolites (glucose, lactate, glycerol, alanine, and glutamine) from each other and from glycogen[4,5].

First, for each pseudo-steady state infusion we calculate the labeled fraction normalized labeling of each metabolite pool, $L_{k\leftarrow i}$, by dividing the fraction of labeled carbon atoms in metabolite k, by the fraction of labeled carbon atoms in the tracer, I, (or of glycogen for the pulse-chase infusion). For example, the normalized labeling of serum glucose from the pulse-chase infusion is denoted as $L_{glc\leftarrow glycogen}$. These values are reported in Table S2 and S3, for the fasted and fed states respectively. Whole body labeling values of glycogen were weighted by their pool size and organ mass (85% liver and 15% muscle).

In each infusion, the normalized labeling a metabolite is the sum of the direct contribution from the traced metabolite and the contributions from secondary contributors: metabolites that also become labeled during the infusion and also contribute label to the metabolite pool of interest. For example, during glucose infusion, lactate can be labeled directly from glucose, or glucose may directly make labeled glycogen which is then broken down into lactate. By performing independent infusions for each potential substrate feeding into a circulating metabolite pool, we determine the contributions from each substrate to each other and to the metabolite pool of interest, enabling us to solve for each input's direct fractional contribution ($f_{k\leftarrow i}$)[5]. For example, if we focus for simplicity on only the contribution of glycogen and circulating lactate to circulating glucose, we get two equations:

$$L_{glc\leftarrow glycogen} = L_{glycogen\leftarrow glycogen} \cdot f_{glc\leftarrow glycogen} + L_{lact\leftarrow glycogen} \cdot f_{glc\leftarrow lact} \quad [S1]$$

$$L_{glc\leftarrow lact} = L_{glycogen\leftarrow lact} \cdot f_{glc\leftarrow glycogen} + L_{lact\leftarrow lact} \cdot f_{glc\leftarrow lact} \quad [S2]$$

Note that the normalized labeling of tracers themselves ($L_{glycogen\leftarrow glycogen}$, $L_{lact\leftarrow lact}$) is by definition 1. To account for *n* potential substrates (with *n* = 5 in our case), we obtain a system of *n* linear equations:

$$\begin{pmatrix} L_{k\leftarrow 1} \\ \vdots \\ L_{k\leftarrow n} \end{pmatrix} = \begin{pmatrix} L_{1\leftarrow 1} & \cdots & L_{n\leftarrow 1} \\ \vdots & \ddots & \vdots \\ L_{1\leftarrow n} & \cdots & L_{n\leftarrow n} \end{pmatrix} \begin{pmatrix} f_{k\leftarrow 1} \\ \vdots \\ f_{k\leftarrow n} \end{pmatrix} \quad [S3]$$

Using this framework, we calculated the direct fractional contributions to each metabolite pool, *k* (circulating glucose, glycogen, circulating lactate, circulating glycerol, circulating alanine, and circulating glutamine), from each of the 5 other metabolite pools, *i*. For each metabolite pool, therefore, we get a system of 5 linear equations. The calculated direct fractional contributions ($f_{k\leftarrow i}$) for each metabolite pool (circulating

metabolites glucose, lactate, glycerol, alanine, and glutamine and tissue glycogen) are provided in Table S2 and S3. To obtain the fraction of each metabolite pool that comes from sources other than what was measured here (e.g. food), we calculated the fraction of k that comes from other sources:

$$f_{k\leftarrow other} = 1 - \sum f_{k\leftarrow i} \quad [S4]$$

Using the direct fractional contributions to a given circulating metabolite and the absolute circulatory flux through that metabolite, we can calculate the absolute flux from each substrate source to that metabolite pool[4]. To do this, we first calculate the carbon-atom total flux in units of nmol C min^{-1} g body $weight^{-1}$ to account for the stoichiometry of carbon atoms between different metabolites. The carbon-atom total flux of metabolite k, $J_{circ,k}$, can be determined by the equation:

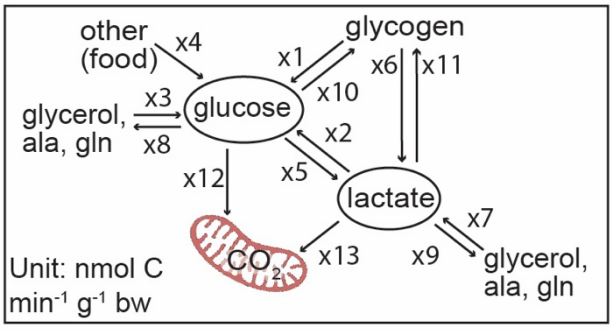
$$J_{circ,k} = \frac{C \cdot R \cdot (1-L)}{L \cdot c_k} \quad [S5]$$

where R is the rate of infusion (nmol molecule /min/g), C is the number of carbon atoms in the tracer molecule, L is the fraction of labeled carbon atoms of the tracer at pseudo-steady state, and c_k is a conversion factor that accounts for the cycling of label through other circulating metabolites that can lead to underestimation of flux[4].

Using $J_{circ,k}$ and the direct fractional contribution ($f_{k\leftarrow i}$) from metabolite pool *i*, we calculate the absolute flux from *i* to *k* ($J_{k\leftarrow i}$):

$$J_{k\leftarrow i} = J_{circ,k} \cdot f_{k\leftarrow i} \quad [S6]$$

Using this method, we determined the production fluxes for glucose, lactate, glycerol, alanine, and glutamine from each other and from glycogen in both the fasted and fed state. Values are provided in Table S2 and S3.



We then set up a flux-balanced model of whole body carbohydrate metabolism.

From the above determined fluxes into the circulating glucose and circulating lactate pools, we can define fluxes x1-x7:

$$\begin{pmatrix} x1 \\ x2 \\ x3 \\ x4 \\ x5 \\ x6 \\ x7 \end{pmatrix} = \begin{pmatrix} J_{glc\leftarrow glycogen} \\ J_{glc\leftarrow lact} \\ J_{glc\leftarrow glycerol} + J_{glc\leftarrow ala} + J_{glc\leftarrow gln} \\ J_{glc\leftarrow food} \\ J_{lact\leftarrow glc} \\ J_{lact\leftarrow glycogen} \\ J_{lact\leftarrow glycerol} + J_{lact\leftarrow ala} + J_{lact\leftarrow gln} \end{pmatrix} \quad [S7]$$

Furthermore, outfluxes from circulating glucose and lactate to circulating glycerol, alanine, and glutamine are also known.

$$\begin{pmatrix} x8 \\ x9 \end{pmatrix} = \begin{pmatrix} J_{glycerol\leftarrow glc} + J_{ala\leftarrow glc} + J_{gln\leftarrow glc} \\ J_{glycerol\leftarrow lact} + J_{ala\leftarrow lact} + J_{glc\leftarrow lact} \end{pmatrix} \quad [S8]$$

The remaining unknown fluxes are outgoing fluxes from circulating glucose and lactate to glycogen and to oxidation. To solve for the fluxes to glycogen, we can use the measurement of glycogen pool size changes over the period of the fasted and fed state infusions. Of the measurements of glycogen pools in the liver, white muscle, and red muscle in the fasted and fed state, only liver in the fed state showed a net production flux of glycogen (Extended data Fig. 9c-d). In addition, in the fasted state the labeling of the glycogen pool from glucose and lactate is small (Table S2), and therefore we assume fluxes to glycogen (x10 and x11) in the fasted state to be zero.

For the fed state, we can use the measured change in the total amount of glycogen in the liver measured over the fed state infusions to calculate the net glycogen production flux.

$$J_{net\ glycogen} = \frac{(C_{glycogen,2} - C_{glycogen,1}) \cdot liver\ weight}{(t_2 - t_1) \cdot body\ weight} \quad [S9]$$

By mass balance, the total gross fed-state glycogen synthesis flux, $J_{gross\ glycogen}$, is equal to the net flux plus the consumption fluxes of glycogen:

$$J_{gross\ glycogen} = J_{net\ glycogen} + x1 + x6 \quad [S2.8]$$

We then need to determine the fraction of total new glycogen synthesis coming from glucose and from lactate. This is given by direct fractional contribution of glucose to glycogen divided by the sum of all direct contributions to glycogen:

$$F_{new\ glycogen\leftarrow glc} = \frac{F_{glycogen\leftarrow glc}}{\sum F_{glycogen\leftarrow i}} \quad [S10]$$

Similarly for lactate:

$$F_{new\ glycogen \leftarrow lact} = \frac{F_{glycogen \leftarrow lact}}{\sum F_{glycogen \leftarrow i}} \quad [S11]$$

Then, the flux of glucose to new glycogen is given by the product of the glycogen synthesis flux and the fraction of new glycogen that comes from glucose:

$$x11 = F_{new\ glycogen \leftarrow glc} \cdot J_{gross\ glycogen} \quad [S12]$$

Similarly, for lactate:

$$x12 = F_{new\ glycogen \leftarrow lact} \cdot J_{gross\ glycogen} \quad [S13]$$

Finally, to solve for the fluxes of glucose and lactate to oxidation, we use mass balance equations for glucose and lactate. The sum of the production fluxes equals the sum of the consumption fluxes.

For glucose:

$$x1 + x2 + x3 + x4 = x5 + x8 + x10 + x12 \quad [S14]$$

For lactate:

$$x5 + x6 + x7 = x2 + x9 + x11 + x13 \quad [S15]$$

The values for each of these fluxes are given in Table S5. Errors were calculated using standard error propagation.

Fasted state data							
quantity of metabolite		metabolite (*k*)					
		glucose	glycogen	lactate	glycerol	alanine	glutamine
carbon-atom circulatory turnover flux (nmol min^{-1} g bw^{-1})	$F_{circ,k}^{atom}$	144 ± 6 n = 9	-	349 ± 26 n = 11	70 ± 4 n = 4	65 ± 2 n = 5	46 ±4 n = 4
labeled fraction (normalized to tracer enrichment)	$L_{k\leftarrow glc}$	-	0.05 ± 0.02 n = 4	0.49 ± 0.02 n = 9	0.08 ± 0.02 n = 4	0.35 ± 0.01 n = 5	0.08 ± 0.01 n = 5
	$L_{k\leftarrow glycogen}$	0.54 ± 0.16 n = 8	-	0.50 ± 0.15 n = 8	0.27 ± 0.04 n = 6	0.38 ± 0.11 n = 8	0.15 ± 0.05 n = 8
	$L_{k\leftarrow lact}$	0.39 ± 0.03 n = 10	0.00 ± 0.00 n = 3	-	0.05 ± 0.03 n = 7	0.73 ± 0.03 n = 9	0.25 ± 0.04 n = 10
	$L_{k\leftarrow glycl}$	0.33 ± 0.03 n = 4	0.00 ± 0.00 n = 3	0.24 ± 0.01 n = 4	-	0.17 ± 0.01 n = 4	0.04 ± 0.00 n = 4
	$L_{k\leftarrow ala}$	0.14 ± 0.03 n = 4	0.00 ± 0.00 n = 3	0.19 ± 0.02 n = 4	0.02 ± 0.00 n = 2	-	0.07 ± 0.01 n = 4
	$L_{k\leftarrow gln}$	0.09 ± 0.01 n = 4	0.03 ± 0.02 n = 4	0.06 ± 0.01 n = 4	0.0 ± 0.00 n = 2	0.02 ± 0.00 n = 4	-
direct fractional contribution	$F_{k\leftarrow glc}$	-	0.04 ± 0.01	0.43 ± 0.01	0.06 ± 0.02	0.00 ±0.00	0.00 ± 0.00
	$F_{k\leftarrow glycogen}$	0.28 ± 0.02	0.00 ± 0.00	0.20 ± 0.08	0.23 ± 0.02	0.01 ± 0.04	0.03 ± 0.02
	$F_{k\leftarrow lact}$	0.30 ± 0.02	0.00 ± 0.00	-	0.02 ± 0.02	0.73 ± 0.02	0.22 ± 0.02
	$F_{k\leftarrow glycl}$	0.24 ± 0.02	0.00 ± 0.00	0.07 ± 0.01	-	0.00 ±0.00	0.00 ± 0.00
	$F_{k\leftarrow ala}$	0.08 ± 0.02	0.00 ± 0.00	0.12 ± 0.01	0.01 ± 0.00	-	0.03 ± 0.01
	$F_{k\leftarrow gln}$	0.06 ± 0.02	0.02 ± 0.01	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	-
	$F_{k\leftarrow other}$	0.03 ± 0.09	0.94 ± 0.01	0.16 ± 0.08	0.68 ± 0.03	0.26 ± 0.05	0.73 ± 0.03
conversion factor	c_k	.78	.99	.73	.77	.96	.93
absolute circulatory flux (nmol C min^{-1} g bw^{-1})	$J_{circ,k}$	867 ± 34	-	1434 ± 106	219 ± 12	251 ± 9	250 ± 21
flux (nmol C min^{-1} g bw^{-1})	$J_{k\leftarrow glc}$	-	-	614 ± 48	13 ± 3	0 ± 0	0 ± 0
	$J_{k\leftarrow glycogen}$	243 ± 74		292 ± 117	50 ± 5	2 ± 11	8 ± 6
	$J_{k\leftarrow lact}$	261 ± 21		-	4 ± 4	183 ± 4	53 ± 5
	$J_{k\leftarrow glycl}$	212 ±19		103 ± 16	-	0 ± 1	6 ± 1
	$J_{k\leftarrow ala}$	66 ± 16		178 ± 21	2 ± 1	-	0 ± 0
	$J_{k\leftarrow gln}$	56 ± 13		14 ± 10	0 ± 0	0 ± 0	-
	$J_{k\leftarrow other}$	29 ± 80		234 ± 120	150 ± 11	66 ± 12	183 ± 8

Table S2. Values from isotope infusions used to calculate production fluxes of circulating metabolites in the fasted state. Related to Figure 6.

Fed state							
quantity of metabolite		metabolite (k)					
		glucose	glycogen	lactate	glycerol	alanine	glutamine
carbon-atom circulatory turnover flux (nmol min^{-1} g bw^{-1})	$F_{circ,k}^{atom}$	348 ± 45 n = 3	-	518 ± 106 n = 3	74 ± 7 n = 3	83 ± 7 n = 3	78 ± 18 n = 3
labeled fraction (normalized to tracer enrichment)	$L_{k\leftarrow glc}$	-	0.52 ± 0.07 n = 3	0.72 ± 0.04 n = 3	0.12 ± 0.02 n = 3	0.34 ± 0.03 n = 3	0.14 ± 0.02 n = 3
	$L_{k\leftarrow glycogen}$	0.02 ± 0.01 n = 6	-	0.26 ± 0.04 n = 6	0.27 ± 0.07 n = 2	0.17 ± 0.01 n = 4	0.09 ± 0.00 n = 4
	$L_{k\leftarrow lact}$	0.16 ± 0.02 n = 7	0.25 ± 0.04 n =3	-	0.04 ± 0.01 n = 6	0.56 ± 0.04 n = 7	0.35 ± 0.01 n = 7
	$L_{k\leftarrow glycl}$	0.08 ± 0.01 n = 3	0.07 ± 0.05 n = 3	0.12 ± 0.01 n = 3	-	0.08 ± 0.01 n = 3	0.10 ± 0.00 n = 3
	$L_{k\leftarrow ala}$	0.05 ± 0.01 n = 3	0.05 ± 0.01 n = 3	0.13 ± 0.02 n = 3	0.01 ± 0.00 n = 3	-	0.02 ± 0.00 n = 3
	$L_{k\leftarrow gln}$	0.02 ± 0.00 n = 3	0.02 ± 0.00 n = 3	0.04 ± 0.01 n = 3	0.02 ± 0.02 n =2	0.07 ± 0.02 n = 3	-
direct fractional contribution	$F_{k\leftarrow glc}$	-	0.37 ± 0.07	0.63 ± 0.02	0.05 ± 0.02	0.00 ±0.00	0.00 ± 0.00
	$F_{k\leftarrow glycogen}$	0.00 ± 0.00	-	0.22 ± 0.02	0.26 ± 0.03	0.03 ± 0.01	0.00 ± 0.01
	$F_{k\leftarrow lact}$	0.13 ± 0.01	0.18 ± 0.03	-	0.00 ± 0.00	0.51 ± 0.02	0.26 ± 0.01
	$F_{k\leftarrow glycl}$	0.05 ± 0.00	0.00 ± 0.00	0.05 ± 0.01	-	0.01 ±0.01	0.7 ± 0.01
	$F_{k\leftarrow ala}$	0.04 ± 0.00	0.02 ± 0.02	0.09 ± 0.01	0.00 ± 0.00	-	0.00 ±0.00
	$F_{k\leftarrow gln}$	0.01 ± 0.00	0.04 ± 0.03	0.01 ± 0.01	0.00 ± 0.00	0.05 ± 0.01	-
	$F_{k\leftarrow other}$	0.78 ± 0.01	0.59 ± 0.11	0.00 ± 0.03	0.68 ± 0.04	0.41 ± 0.03	0.67 ± 0.01
conversion factor	c_k	.88	.88	.79	.86	.95	.93
absolute circulatory flux (nmol C min^{-1} g bw^{-1})	$J_{circ,k}$	2387 ± 307	-	1970 ± 402	235 ± 13	290 ± 23	419 ± 97
flux (nmol C min^{-1} g bw^{-1})	$J_{k\leftarrow glc}$	-	-	1236 ± 256	13 ± 5	0 ± 0	0 ± 0
	$J_{k\leftarrow glycogen}$	0 ± 0		434 ± 95	62 ±8	8 ± 4	0 ± 2
	$J_{k\leftarrow lact}$	312 ± 40		-	0 ± 0	146 ± 13	108 ± 25
	$J_{k\leftarrow glycl}$	118 ± 15		98 ± 28	-	2 ± 2	29 ± 7
	$J_{k\leftarrow ala}$	85 ± 11		170 ± 43	0 ± 0	-	0 ± 0
	$J_{k\leftarrow gln}$	22 ± 3		25 ± 14	0 ± 0	16 ± 4	-
	$J_{k\leftarrow other}$	1850 ± 307	-	8 ± 67	161 ± 12	117 ± 12	282 ± 65

Table S3. Values from isotope infusions used to calculate production fluxes of circulating metabolites in the fed state. Related to Figure 6.

quantity	value
$J_{net\ glycogen}$ (nmol C g^{-1} min^{-1})	125 ± 48
$F_{new\ glycogen \leftarrow glc}$ (fraction of new glycogen from glucose)	0.61 ± 0.14
$F_{new\ glycogen \leftarrow lact}$ (fraction of new glycogen from lactate)	0.29 ± 0.06

Table S4. Values used the calculate glycogen synthesis fluxes. Related to Figure 6.

fluxes (nmol C min^{-1} g body $weight^{-1}$)			
	direction	Fasted	fed
x1	glycogen to glucose	243 ± 74	0 ± 0
x2	lactate to glucose	261 ± 21	312 ± 40
x3	glycerol, alanine, glutamine to glucose	333 ± 28	225 ± 19
x4	other to glucose	29 ± 80	1850 ± 307
x5	glucose to lactate	614 ± 48	1236 ± 256
x6	glycogen to lactate	292 ± 117	434 ± 95
x7	glycerol, alanine, glutamine to lactate	295 ± 28	293 ± 52
x8	glucose to glycerol, alanine, glutamine	13 ± 3	13 ± 5
x9	lactate to glycerol, alanine, glutamine	241 ± 10	254 ± 28
x10	glucose to glycogen	-	402 ± 271
x11	lactate to glycogen	-	191 ± 127
x12	glucose to CO_2	239 ± 60	735 ± 485
x13	lactate to CO_2	507 ± 64	1205 ± 310

Table S5. Modeled whole-body fluxes. Related to Figure 6.

References

Hui, S., Cowan, A.J., Zeng, X., Yang, L., TeSlaa, T., Li, X., Bartman, C., Zhang, Z., Jang, C., Wang, L., et al. (2020). Quantitative Fluxomics of Circulating Metabolites. Cell Metabolism.