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Supplementary information

Circulating metabolite homeostasis achieved through mass action

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SUPPLEMENTARY INFORMATION.

Supplementary Table S1. Akaike information criterion (AIC) comparison of different quantitative models. Data from **Fig. 2**, **Fig. 4A** (refed state), **Fig. 4B** (lower panel), **Fig. 5** (top panel), and **Fig. S5B** were analyzed with GraphPad Prism 8 to compare different models to the default model in the leftmost column heading. $\Delta AICc = AICc_{default} - AICc_{alternative}$, and accordingly negative ΔAICc values reflect the default being preferred while positive values reflect the alternative being preferred. Differences greater than +2 reflect the alternative being significantly preferred and are highlighted in red.

Supplementary Table S2. Different bolus doses are cleared with similar elimination constants. Mean ± SD, n=3 mice.

Supplementary Table S3. Fasting serum concentrations of essential amino acids reflect the surplus flux generated by protein degradation (beyond that needed for protein synthesis) and the amino acid's propensity for TCA oxidation (*b*). R_d, unperturbed consumption flux (equivalent to F_{circ}) from Hui et. al. ***(Error! Reference source not found.)***; *S*, protein synthesis flux (sum of observed tissue protein synthesis rates, corrected for the relevant amino acid's abundance in protein), mean ± SD, n=4 mice per time point for calculation; TCA oxidation flux $T_{SS} = R_d - S$; *b*, slope of relationship between consumption flux and circulating concentration (best-fitted value from linear regression in **Fig. 2**); $[M]_{SS}$, steady-state unperturbed essential amino acid concentrations calculated by the equation (10). Fasting serum essential amino acid concentrations were experimentally measured using LC-MS and isotopic standards. Mice were fasted from 9 AM to 5 PM (8 h fasting) and blood was taken at 5 PM. Mean ± SD, n=6 mice. Note that the calculated circulating concentrations based on equation (10) ($[M]_{SS}$) generally agree well with the experimental measurements.

Supplementary Table S4. Flux calculation for Figure 6F.

Supplementary Table S5. Composition of high, normal, and low protein diets.

Supplementary Table S6. Relative amino acid levels across dietary conditions. P-values were calculated by two-tailed Student's t-test without correction for multiple comparison. Amino acids with $p<0.01$ are highlighted in red.

Supplementary Table S7. Doses used for intravenous bolus injections and infusions for each tracer.

For Figure 2:

ΔAICc compared to linear regression with fixed y-intercept	Linear regression	Michaelis-Menten
Valine infusion	-3.93	-6.90
Phenylalanine infusion	-4.09	-1.98
Lysine infusion	-3.67	-0.86
Methionine infusion	12.00	25.46

ΔAICc compared to linear regression	Michaelis-Menten
3-HB infusion	1.33
Citrate infusion	-2.77
Alanine infusion	-0.78
Serine infusion	8.65

For Figure 4A:

ΔAICc compared to linear regression with fixed slope and y-intercept	Linear regression
Valine infusion	-22.10
Lysine infusion	-6.51
Serine infusion	-24.90
3-HB infusion	-24.30
Alanine infusion	-10.53
Citrate infusion	-4.486
Methionine infusion	14.27

For Figure 4B:

ΔAICc compared to linear regression with fixed y-intercept	Linear regression	Michaelis-Menten
Valine infusion with BT2	3.62	10.01

For Figure 5:

ΔAICc compared to linear regression with fixed y-intercept	Linear regression	Michaelis-Menten
Liver	-0.20	-1.15
Kidney	-4.61	-2.46
Pancreas	6.31	10.14

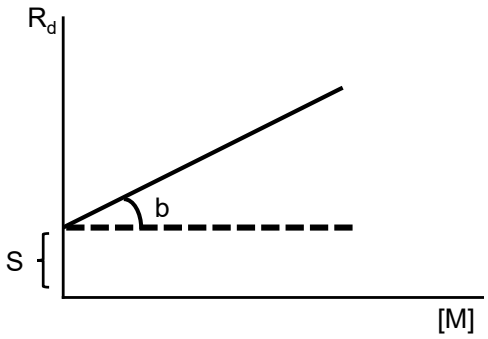
For Figure S5B:

ΔAICc compared to linear regression with fixed y-intercept	Linear regression	Michaelis-Menten
Brain	-0.48	-0.21
Heart	-4.05	-4.67
Lung	-4.83	-5.44
Jejunum	0.30	1.58
Spleen	2.87	4.98
Quadriceps	-1.90	2.3

Supplementary Table S1. Akaike information criterion (AIC) comparison of different quantitative models.

Elimination constant γ (min^{-1})	Low dose	Medium dose	High dose
Valine	0.077 ± 0.005	0.071 ± 0.009	0.069 ± 0.009
Methionine	0.064 ± 0.010	0.049 ± 0.025	0.061 ± 0.005
Leucine	0.046 ± 0.012	0.073 ± 0.020	0.064 ± 0.013
Lysine	0.042 ± 0.014	0.046 ± 0.026	0.056 ± 0.005

Supplementary Table S2. Different bolus doses are cleared with similar elimination constants.



$$[M_{SS}] = (R_d - S)/b = T_{SS}/b$$

Parameters (units)	Valine	Methionine	Phenylalanine	Lysine
R_d (nmol/g bodyweight/min)	9.6±0.4	3.9±1.6	5.9±0.8	9.3±1.8
S (nmol/g bodyweight/min)	6.4±0.5	1.8±0.1	3.7±0.3	6.4±0.5
T_{SS} (nmol/g bodyweight/min)	3.2±0.6	2.1±1.6	2.2±0.9	2.9±1.9
b (min^{-1})	0.018	0.049	0.055	0.024
$[MSS]_\infty$ (μM)	178±33	43±33	40±16	121±79
Fasting serum concentration (μM)	167±14	63±9	73±7	282±46

Supplementary Table S3. Fasting serum concentrations of essential amino acids reflect the surplus flux generated by protein degradation (beyond that needed for protein synthesis) and the amino acid's propensity for TCA oxidation (*b*).

Valine production and consumption fluxes	Measurements and calculations	Figure	Mean±s.d. (nmol/g bodyweight/min)
Fasted-state production from protein degradation (P)	Tracer infusion (Hui et al. (*18*))	S8	10.2±1.6
Fasted-state consumption by protein synthesis (S)	Sum of observed tissue protein synthesis rates, corrected for valine fraction in protein	6B	6.2±0.5
Fasted-state consumption by TCA cycle (T)	Flux balance: T = P – S	-	4.0±1.7
Fed-state production from dietary protein (D)	Flux calculated by: $\frac{food\ intake * valine\ percentage}{infusion\ duration * bodyweight}$	6E	8.9
Fed-state production from protein degradation (P)	Double tracer infusion: $P = R_{inf} * E_{[U\text{-}13C]\text{-}Val} / E_{[U\text{-}2H]\text{-}Val}$	6D	5.0±0.3
Fed-state consumption by protein synthesis (S)	Sum of observed tissue protein synthesis rates, corrected for valine fraction in protein	6B	6.6±0.3
Fed-state consumption by TCA cycle (T)	Flux balance: T = D + P – S	-	7.2±0.4

Supplementary Table S4. Flux calculation for Figure 6F.

Diets	Protein (kcal%)	Carbohydrate (kcal%)	Fat (kcal%)	Cellulose (gm%)	Inulin (gm%)	Total Calories (kcal/gm)
Low Protein	10	75	15	7	2.3	4.084
Normal Protein	20	65				
High Protein	40	45				

Supplementary Table S5. Composition of high, normal, and low protein diets.

		Group Mean		*t*-test
		log_2 (HP/NP)	log_2 (LP/NP)	*p*-value (HP vs LP)
Fed	Valine	1.22±0.72	0.24±0.30	$2.7x10^{-7}$
	Isoleucine	1.12±0.66	0.23±0.43	$3.4x10^{-6}$
	Leucine	1.24±0.76	0.24±0.44	$2.2x10^{-6}$
	Phenylalanine	0.55±0.29	0.13±0.24	$2.0x10^{-5}$
	Tryptophan	0.99±0.17	0.39±0.55	$1.1x10^{-2}$
	Methionine	0.52±0.37	0.33±0.32	$7.0x10^{-4}$
	Lysine	0.38±0.16	0.17±0.24	$1.0x10^{-3}$
	Threonine	0.43±0.50	0.41±0.43	$2.8x10^{-3}$
	Histidine	0.16±0.07	0.09±0.18	$1.2x10^{-2}$
	Asparagine	0.41±0.07	0.31±0.38	$2.7x10^{-2}$
	Arginine	0.35±0.13	0.24±0.23	$8.3x10^{-2}$
	Glycine	-0.29±0.02	0.13±0.22	$1.2x10^{-2}$
	Glutamine	0.07±0.36	0.13±0.23	$1.8x10^{-2}$
	Alanine	0.01±0.17	0.12±0.22	$8.9x10^{-2}$
	Tyrosine	1.45±0.09	0.46±0.41	$3.1x10^{-4}$
	Proline	0.58±0.50	0.26±0.39	$2.1x10^{-4}$
	Serine	0.18±0.04	0.25±0.34	$2.4x10^{-1}$
	Glutamate	0.08±0.04	0.23±0.14	$3.8x10^{-1}$
	Aspartate	0.14±0.16	0.21±0.16	$4.2x10^{-1}$
		Group Mean		*t*-test
		log_2 (HP/NP)	log_2 (LP/NP)	*p*-value (HP vs LP)
Fasted	Valine	0.04±0.42	0.17±0.19	$1.1x10^{-3}$
	Leucine	0.01±0.39	0.18±0.23	$5.9x10^{-3}$
	Isoleucine	0.05±0.47	0.17±0.22	$1.0x10^{-3}$
	Phenylalanine	-0.15±0.24	0.14±0.12	$1.5x10^{-1}$
	Tryptophan	-0.001±0.48	0.15±0.44	$2.4x10^{-2}$
	Methionine	-0.01±0.24	0.18±0.27	$1.7x10^{-1}$
	Lysine	-0.06±0.24	0.28±0.16	$3.4x10^{-2}$
	Threonine	-0.05±0.29	0.15±0.31	$7.0x10^{-2}$
	Histidine	-0.20±0.15	0.16±0.09	$8.9x10^{-4}$
	Asparagine	-0.34±0.27	0.08±0.29	$3.2x10^{-1}$
	Arginine	-0.32±0.32	0.31±0.39	$8.6x10^{-3}$
	Glycine	-0.34±0.18	0.09±0.21	$7.7x10^{-2}$
	Glutamine	-0.26±0.01	0.12±0.10	$3.0x10^{-3}$
	Alanine	-0.24±0.15	0.25±0.30	$3.0x10^{-1}$
	Tyrosine	0.19±0.56	0.09±0.51	$4.4x10^{-3}$
	Proline	-0.20±0.16	0.21±0.11	$3.5x10^{-1}$
	Serine	-0.13±0.19	0.16±0.26	$3.3x10^{-1}$
	Glutamate	0.06±0.02	0.33±0.24	$4.2x10^{-1}$
	Aspartate	-0.02±0.04	0.26±0.16	$4.5x10^{-1}$

Supplementary Table S6. Relative amino acid levels across dietary conditions.

A

Tracer	Dose (μmol/g bodyweight)
Glucose	2, 4, 8
Valine	0.2, 0.4, 1
Leucine	0.24, 0.48, 1
Methionine	0.1, 0.2, 0.5
Lysine	0.2, 0.4, 1
Alanine	0.5, 1, 2
3-HB	0.1, 0.2, 0.4
Citrate	0.15, 0.3, 0.45
Serine	0.25, 0.5, 1

B

Tracer	Infusion rate (nmol/g bodyweight/min)
Glucose	20, 40, 60, 80
Valine	1, 2, 5, 10, 20, 30
Lysine	1, 2, 5, 8, 10, 15, 20
Phenylalanine	1, 8, 10, 15, 20, 25
Methionine	1, 5, 10
Alanine	5, 10, 15, 20, 60, 150, 200
3-HB	4, 20, 40, 60, 80, 100
Citrate	3, 6, 20, 30, 40
Serine	2.5, 5, 10, 20, 40, 50, 60

Supplementary Table S7. Doses used for intravenous bolus injections and infusions for each tracer.