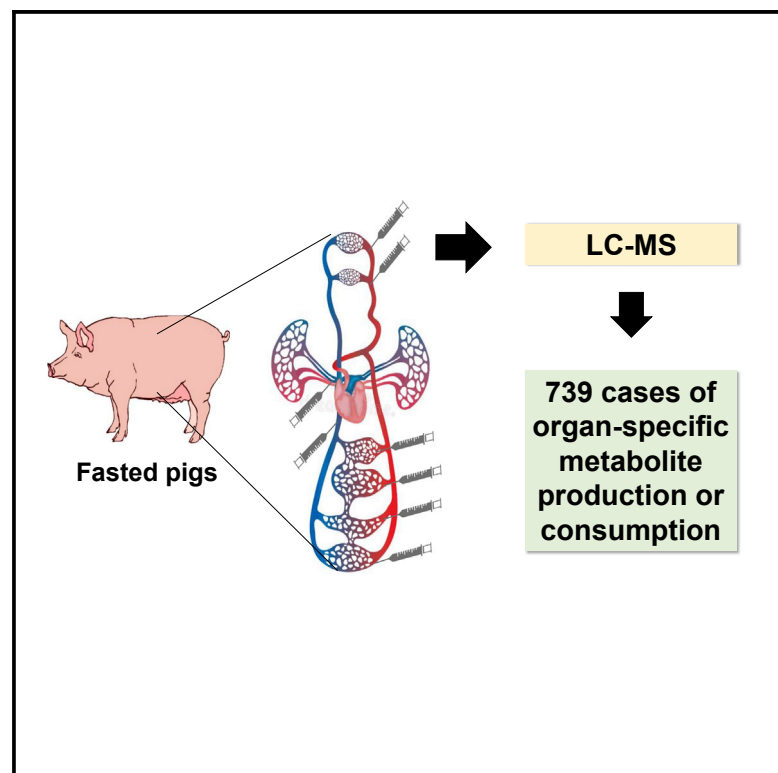


Cell Metabolism

Metabolite Exchange between Mammalian Organs Quantified in Pigs

Graphical Abstract



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In Brief

Using metabolomics in the arterial blood and draining veins of 11 organs in fasted pigs, Jang et al. map more than 700 cases of organ-specific metabolite production or consumption. The resulting data resource provides a blueprint for the integrative function of mammalian metabolism.

Highlights

- A systems-level atlas of organ-specific metabolite production and consumption
- 700 quantitative measurements of organ-specific uptake or excretion
- Kidneys burn circulating citrate and net-oxidize lactate to pyruvate
- Liver preferentially consumes unsaturated over saturated fatty acids

Metabolite Exchange between Mammalian Organs Quantified in Pigs

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SUMMARY

Mammalian organs continually exchange metabolites via circulation, but systems-level analysis of this shuttling process is lacking. Here, we compared, in fasted pigs, metabolite concentrations in arterial blood versus draining venous blood from 11 organs. Greater than 90% of metabolites showed arterial-venous differences across at least one organ. Surprisingly, the liver and kidneys released not only glucose but also amino acids, both of which were consumed primarily by the intestine and pancreas. The liver and kidneys exhibited additional unexpected activities: liver preferentially burned unsaturated over more atherogenic saturated fatty acids, whereas the kidneys were unique in burning circulating citrate and net oxidizing lactate to pyruvate, thereby contributing to circulating redox homeostasis. Furthermore, we observed more than 700 other cases of tissue-specific metabolite production or consumption, such as release of nucleotides by the spleen and TCA intermediates by pancreas. These data constitute a high-value resource, providing a quantitative atlas of inter-organ metabolite exchange.

INTRODUCTION

Converting environmental nutrient inputs, whose availability is often unreliable, into energy and biomass is a fundamental chal-

lenge of life. Unicellular organisms, like bacteria or yeasts, face this challenge in each cell individually. An important advantage of multi-cellularity is the ability of cells to work in concert to achieve metabolic homeostasis (Chantranupong et al., 2015). This in turn allows organs to develop specialized metabolism, which is adapted to their particular biological function, with metabolites produced in one organ transmitted to another organ via the circulatory system.

The biological significance of metabolite exchange has been recognized since the discovery of the Cori cycle (Cori, 1981), in which lactate made by skeletal muscle is converted into glucose by the liver. Since then, other examples of metabolite exchange have been elucidated (e.g., alanine cycle between the liver and muscle and glutamate-glutamine cycle between neurons and astrocytes) (Felig, 1973; Bak et al., 2006). However, these findings are limited to only a few metabolites and organs. Comprehensive mapping of inter-organ metabolite exchange has not yet been achieved. Understanding the production and consumption sites of circulating metabolites is important because it can facilitate diagnosis or treatment of diseases involving either the organ or metabolite. In addition, it is key to understanding the integrative function of mammalian metabolism.

Each organ is fed by arterial blood and drained by venous blood (with the liver also fed by the portal vein from the intestine). Measurement of arterio-venous (AV) concentration differences is a well-established approach for quantification of organ-specific metabolite production and consumption (Cantarow and Richiuti, 1934; Hickam et al., 1948; Ivanisevic et al., 2015). To investigate metabolite exchange between organs, we performed metabolomics in the arterial blood, the organ-specific draining veins, and the urine of pigs. We chose pigs because their diet and metabolism are similar to humans (Perleberg et al., 2018;

Context and Significance

Different organs in the body have specific preferences for their fuel source and produce particular waste products. For example, lactate is known to be produced by muscle and converted to glucose by liver. Researchers at Princeton University and the University of Pennsylvania used state of the art metabolomics of blood going in and coming out of eleven organs in the pig to identify over 700 cases of organ-specific metabolite production or consumption. The data is relevant also to humans, as pigs have similar diet and metabolism. Besides providing a useful resource of inter-organ metabolite exchange, their analysis highlighted unexpected findings that can have medical relevance, such as the preferential consumption of heart-healthy unsaturated fatty acids, as opposed to atherogenic saturated fatty acids, by the liver.

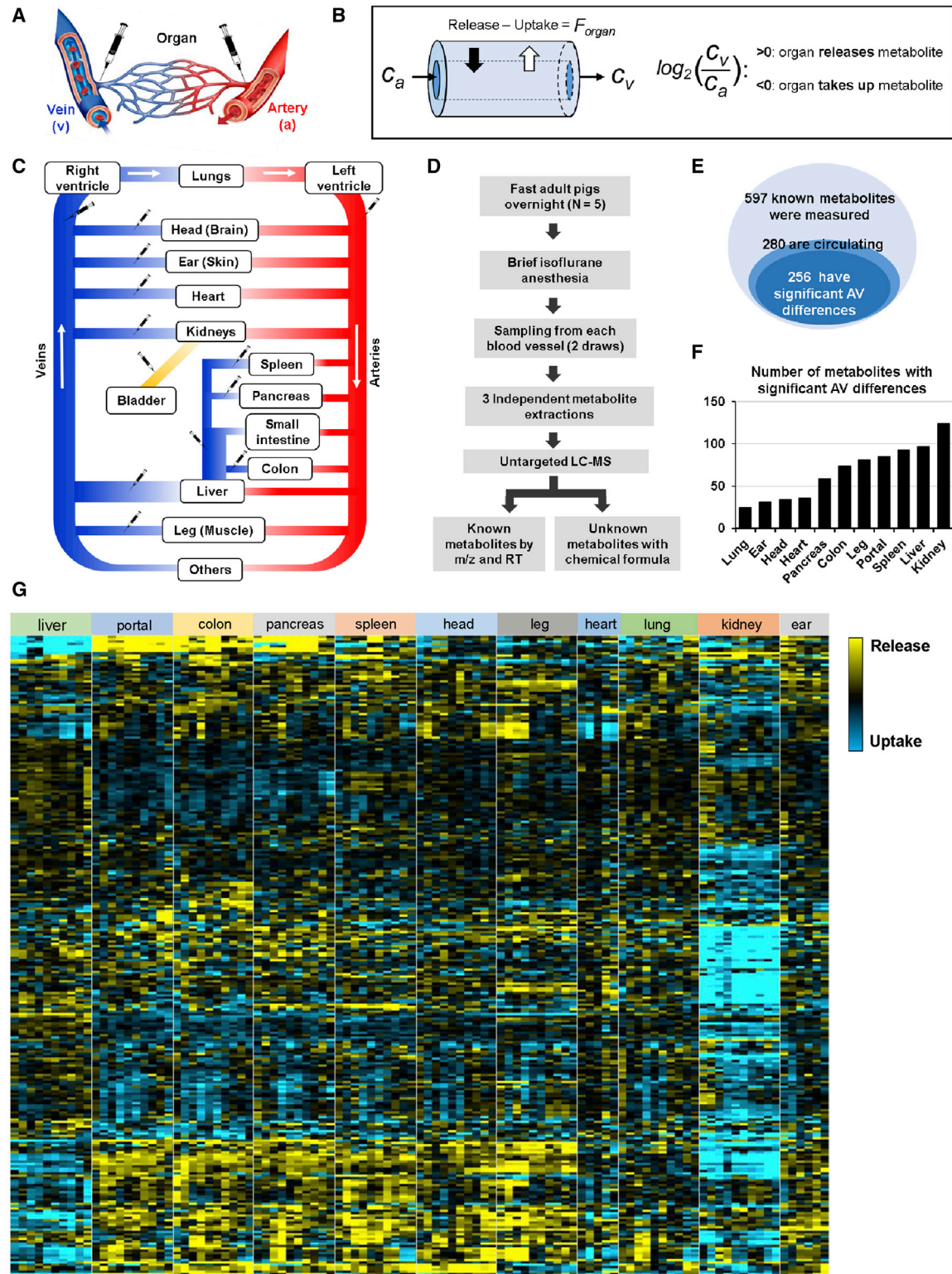


Figure 1. The Landscape of Metabolite Exchange between Organs

(A) AV difference measurements were achieved by sampling blood from the arterial circulation and organ-specific draining veins of fasting, anesthetized pigs. (B) The difference between metabolite production and consumption by an organ (the net flux, F_{organ}) results in enrichment or depletion of the metabolite in the exiting venous blood relative to entering arterial blood (concentrations C_v and C_a). This concentration difference is measured by LC-MS and reported as a log-fold change.

(legend continued on next page)

Prather et al., 2008). In addition, their large size ensures adequate blood volume and results in 11 different draining veins being sufficiently large to sample reliably. These include veins draining many major metabolic organs (liver, kidney, intestine, colon, spleen, pancreas, heart, lung, and brain). Ear vein was selected because it is enriched in draining from skin. No accessible sampled vein selectively drains from adipose, with the femoral vein draining both adipose and major leg muscles.

To confirm the generality of key observations from the pig AV concentration difference measurements, we utilized *in vivo* isotope tracing in mice. In this manner, we verified several key insights from the pig AV data, such as use of citrate to fuel the kidney and preferential hepatic catabolism of unsaturated over saturated fatty acids. This holistic analysis of metabolite exchange revealed sources and sinks of the circulating metabolome, resulting in a fundamental dataset containing over 700 significant cases of organ-specific metabolite production or consumption. Collectively, this resource illuminates the metabolic roles of mammalian organs.

RESULTS AND DISCUSSION

General Features of Metabolite Exchange

To obtain arterial and venous blood for measuring metabolite exchange, overnight-fasted adult pigs (~50 kg) were briefly anesthetized and blood was drawn from the arterial circulation and organ-specific draining veins (Figure 1A). For each vascular bed, we calculated the \log_2 ratio of metabolite abundance in the draining vein (C_v) relative to the systemic arterial blood (C_a) (Figure 1B). Values of $\log_2(C_v/C_a)$ greater than zero indicate net release, whereas values less than zero indicate net uptake. Because the liver is fed both by the hepatic artery (22%) and the portal vein (78%) (Eipel et al., 2010), we used a weighted average of these two inputs to determine metabolite concentrations feeding the liver. Multiple organs drain into the hepatic portal vein (e.g., spleen, pancreas, and intestines), which we collectively refer to as the other visceral organs (Figure 1C).

A challenge with measuring metabolite exchange based on AV concentration differences is that, due to the speed of vascular flow, biologically important uptake and excretion fluxes may cause only small AV differences, especially for abundant metabolites. Accordingly, precise measurements are required. To enhance measurement precision, blood was drawn from each vessel twice, and each resulting serum sample was independently extracted and measured by liquid chromatography-mass spectrometry (LC-MS) 3 times. Figures show the median value of each independent blood draw. Statistical analyses were conducted using a single value for each pig (the average of the two independent blood draws), with n = the number of pigs (Figure 1D).

Our metabolomics methods measure ~600 known small molecule metabolites, whose identities were confirmed by exact mass and retention time match to authenticated standards. Among these, 280 metabolites were detected in the pig serum (Figure 1E), with 91% showing statistically significant AV differences for at least one organ (Figures 1E and 1F). In total, we detected 739 statistically significant cases of organ-specific metabolite production or consumption (Data 1; for selected examples, see Table 1). These were roughly equally distributed across abundant and less abundant metabolites (Figure S1).

Heatmap analysis revealed a few overarching trends (Figure 1G; Data 2 and 3). Consistent with their key role in waste removal, the kidneys clear ~50% of circulating metabolites, some of which are made by nearly every other organ (note the long blue column in the heatmap). Many other metabolites are cleared by the liver (top and bottom on heatmap), with the strongest changes occurring in microbiome-derived products made in the intestine (top of heatmap). Conversely, the liver also releases many metabolites, including many amino acids, which are consumed by the rest of the body, especially the other visceral organs (middle of heatmap).

In addition to these general patterns, certain metabolites have specific production and consumption sites (distinctive thin yellow or blue lines in the heatmap). Table 2 summarizes the three most substantially produced and consumed metabolites for each organ, taking into account both the statistical significance and AV-fold change. Table S1 summarizes sinks and sources of each metabolite.

Untargeted metabolomics also identified many unannotated ions with significant AV differences. Among them, we assigned chemical formulas to 55 putative metabolites (after exclusion of adducts and isotopic variants), based on accurate mass, natural isotopic distribution, and MS/MS (Figure S2). Examples include $C_{20}H_{34}O_9$, produced only by the leg and $C_7H_7NO_4$, produced by the liver. The existence of these organ-specific production and consumption patterns provides a particular motivation to identify these compounds.

Kidney Metabolism

The primary role of the kidneys is waste excretion. This is achieved by filtration of the blood and reabsorption of salt and circulating nutrients. Kidneys also produce glucose and other specific metabolites, including glycoamine (creatine precursor) and serine (Figure 2A). Patients with the kidney failure display significantly lower circulating levels of glycoamine (Kikuchi et al., 1981). We also identified guanosine, allantoate, and phenylacetate as additional kidney-specific metabolic products, which may be useful diagnostic markers for renal dysfunction.

(C) Schematic of the circulatory system. Syringes indicate blood sampling sites.

(D) Workflow.

(E) Venn diagrams of measured metabolites, circulating metabolites, and metabolites with significant AV differences.

(F) Number of metabolites with significant AV differences for each organ.

(G) Heatmap of AV differences of circulating metabolites across organs. Yellow indicates release and blue indicates uptake. Each data point indicates the median of three technical replicates, with independent duplicate blood draws from the same pig shown separately (n = 5 pigs and 10 data points for most organs; only a single blood draw per pig was performed in the heart, and only 3 pigs were used for the ear vein sampling). Leg, head, and ear primarily reflect skeletal muscle, brain, and skin metabolism, respectively.

See also Figures S1, S2, and Data 1, 2, and 3.

Table 1. Selected Examples of Organ-Specific Metabolite Production and Consumption

Organ	Exemplary Discovery	Key Evidence
Liver	clears unsaturated fatty acids	compared to the most abundant saturated fatty acids, oleate (C18:1) and linoleate (C18:2) show greater uptake and TCA contribution
	produces amino acids	significant release of amino acids
Intestine	consumes glucose and amino acids	greatest absolute uptake flux, of any organ, of both glucose and amino acids
Pancreas	produces TCA intermediates	significant release of citrate, ketoglutarate, succinate, fumarate, and malate
Spleen	produces nucleosides	significant release of cytidine, deoxycytidine, deoxyuridine, guanosine, inosine, thymidine, uridine, and xanthosine
	produces unsaturated very long chain fatty acids	significant release of C22:1, C22:2, C22:3, C22:4, C22:5, C22:6, C24:1, C24:2, C24:3, C24:4, and C24:5
Brain	produces acetate	>2× increase in acetate in jugular vein blood
Leg muscle	consumes short chain acylcarnitines	significant uptake of C2:0, C3:0, C4:0, C5:0, and C5:1 carnitines
	produces long chain acylcarnitines	significant release of C8:0, C10:0, C12:0, C12:1, C14:1, C14:2, C16:0, C16:1, C18:1, C18:2, and C20:4 carnitines
Heart	consumes long chain fatty acids	significant uptake of C16:0, C16:1, C18:0, C18:1, C18:2, C20:1, C20:2, C22:4, C24:0, and C24:1
Lung	produces saturated very long chain fatty acids	significant release of C22:0 and C24:0
Kidney	consumes citrate	only organ with significant citrate uptake; TCA contribution from citrate >10× higher than any other organ
	maintains circulating pyruvate/lactate ratio	significant increase in pyruvate relative to lactate in renal vein blood
	produces amino acids	significant release of amino acids
	consumes medium and long chain acylcarnitines	significant uptake of C5:0, C6:0, C8:0, C10:0, C10:1, C12:0, C12:1, C14:0, C14:1, C14:2, and C16:1 (without release into urine)

Among the much larger set of metabolites that are cleared by the kidneys, we observed a strong correlation between the metabolite's absolute concentration in the blood, and its reabsorption efficiency, with more abundant metabolites being more effectively reabsorbed (Figure 2B). Notable exceptions to this trend were classical metabolic waste products such as uric acid and creatinine. For this analysis, we omitted fatty acids and acylcarnitines, which are relatively hydrophobic and were retained in blood regardless of their concentrations (Figure S3A), perhaps because they are bound to circulating albumin and thus never filtered. The correlation between reabsorption efficiency and plasma concentration of water-soluble metabolites is consistent with an evolutionary drive to avoid wasting abundant circulating metabolites. It also suggests that renal reabsorption efficiency is a major determinant of circulating metabolite levels.

The kidneys' continuous reabsorption against metabolite concentration gradients demands substantial energy. What feeds the kidneys? Strikingly, kidneys were distinguished by taking up circulating TCA (tricarboxylic acid) cycle intermediates, the most abundant of which is citrate (Figure 2C). Indeed, citrate showed the fourth largest renal uptake after lactate, uric acid, and glutamine (Figure S3B). To determine the fate of these species, we intravenously infused a tracer amount of ¹³C-labeled citrate, malate, or succinate into mice. Measurement of tissue TCA intermediates revealed a large contribution of citrate to the kidney TCA cycle (Figure 2D). This is an intrinsic aspect of renal metabolism, as the infusion was non-perturbative (10% circulating citrate labeling, no detectable change in circulating citrate concentra-

tion; Figure S3C). Malate and succinate did not make a comparable contribution in kidney, nor did any of these make a major contribution in other organs (Figure S3D). The quantitative contribution of circulating citrate to the kidney TCA cycle was 20%, in the same range as glutamine and lactate (Hui et al., 2017). Thus, the kidneys are exceptional in using circulating citrate as fuel.

The ability of the kidney to catabolize citrate has been known for some time (Baruch et al., 1975; Hamm, 1990; Simpson, 1967), although the quantitative extent and uniqueness of this metabolic function had not been previously determined. The innate capacity of the kidneys to catabolize citrate may explain key features of kidney cancer, whose rate is steadily increasing (Chow et al., 1999). Mutations in enzymes of the left side of the TCA cycle (succinate dehydrogenase and fumarate hydratase) are oncogenic (Sullivan et al., 2013). These mutations cause cancer through epigenetic mechanisms that are not kidney-specific—inhibition of α -ketoglutarate-dependent demethylases—but the mutations nevertheless occur primarily in cancers of the kidneys (Raimundo et al., 2011). This may reflect the mutations being detrimental in other tissues, as they block endogenous citrate synthesis, but not in the kidneys, which can consume citrate from the circulation. While TCA mutations are relatively rare, citrate usage may more generally impact the properties of renal cancer. Clear cell renal cell carcinoma is exceptionally lipid laden and shows overexpression of ATP citrate lyase, which converts citrate into lipid precursors (Teng et al., 2018). Thus, high citrate uptake may shape both the mutational landscape and pathophysiology of kidney cancer (Ricketts et al., 2018).

Table 2. Top Three Metabolites Produced and Consumed by Each Organ in Pigs

Organ	Production		Consumption	
	Metabolite	Log2 (V/A)	Metabolite	Log2 (V/A)
Liver	glutamate	0.64 ± 0.11	bile acids (5)	-2.89 ± 0.19
	triethanolamine	0.49 ± 0.17	phenylpropionic acid (2)	-2.29 ± 0.12
	acetoacetate	0.38 ± 0.09	short-chain fatty acids (3)	-2.02 ± 0.83
Portal (intestine)	bile acids (6)	3.28 ± 0.21	2-methylhippuric acid	-0.69 ± 0.15
	phenylpropionic acid (2)	2.84 ± 0.32	glucose	-0.31 ± 0.05
	short-chain fatty acids (3)	2.82 ± 1.15	glutamine	-0.28 ± 0.02
Colon	short-chain fatty acids (3)	4.65 ± 1.21	2-methylhippuric acid	-0.60 ± 0.21
	lithocholic acid	4.04 ± 1.10	5-hydroxylysine	-0.41 ± 0.05
	phenylpropionic acid (2)	3.42 ± 0.48	glucose	-0.39 ± 0.05
Pancreas	xanthine	1.05 ± 0.26	5-hydroxylysine	-0.79 ± 0.19
	capryloyl glycine	0.51 ± 0.09	N-carbamoylsarcosine	-0.39 ± 0.09
	TCA intermediates (5)	0.36 ± 0.17	amino acids (8)	-0.36 ± 0.01
Spleen	O-phosphorylethanolamine	1.11 ± 0.22	adenosine	-0.61 ± 0.14
	nucleosides (9)	0.52 ± 0.03	dihydroxymandelic acid	-0.33 ± 0.07
	C22 and C24 very long-chain fatty acids (11)	0.35 ± 0.008	C5 acylcarnitine	-0.26 ± 0.02
Head (brain)	synephrine	1.89 ± 0.68	dihydroxymandelic acid	-0.38 ± 0.12
	gluconolactone and gluconate	1.66 ± 0.03	2-methylhippuric acid	-0.34 ± 0.11
	acetate	1.46 ± 0.39	glutamate	-0.30 ± 0.09
Leg (muscle)	hypotaurine	0.69 ± 0.12	glutamate	-1.41 ± 0.33
	branched chain hydroxyl acids (2)	0.65 ± 0.12	ketone bodies (2)	-0.58 ± 0.14
	medium and long-chain acylcarnitines (11)	0.57 ± 0.02	short-chain acylcarnitines (5)	-0.36 ± 0.05
Lung	2-phenylpropionic acid	0.48 ± 0.14	5-keto-D-gluconic acid	-0.31 ± 0.07
	aconitate	0.26 ± 0.03	kynurenate	-0.22 ± 0.03
	C22:0 and C24:0 fatty acids	0.24 ± 0.02	3-hydroxyanthranilic acid	-0.17 ± 0.02
Kidney	glycocyamine	1.87 ± 0.12	N-formyl-L-methionine	-2.66 ± 0.32
	serine	0.73 ± 0.12	medium-chain acylcarnitines (4)	-2.61 ± 0.19
	allantoate	0.53 ± 0.12	N-acetyl amino acids (9)	-1.27 ± 0.87
Heart	hypotaurine	0.34 ± 0.11	3-phenylpropionic acid	-0.71 ± 0.24
	glutamate	0.26 ± 0.04	unsaturated long-chain fatty acids (11)	-0.53 ± 0.06
	biotin	0.25 ± 0.06	hydroxyindoleacetic acid	-0.47 ± 0.12
Ear (skin)	guanine	0.82 ± 0.19	hydroxyhippuric acid	-0.35 ± 0.08
	taurine	0.53 ± 0.08	indole metabolites (2)	-0.23 ± 0.02
	long-chain acylcarnitines (3)	0.20 ± 0.04	serine	-0.15 ± 0.01

Ranking is based on multiplying the log2 (t value) and log2 (V/A) to reflect both statistical significance and fold change. Numbers in parentheses refer to the number of metabolites in that category showing statistically significant AV differences across the indicated organ. All AV differences included in the table are statistically significant (FDR < 0.05).

Glucose and Lactate

To maintain circulating metabolite homeostasis, for each metabolite, total production and consumption across all organs must balance (accounting for net uptake from the diet and net excretion to urine and feces) (Figure 3A). For an organ i , the net flux (F_i) can be calculated from the AV difference data and literature data for absolute metabolite concentrations and regional blood flow:

$$F_i = Q_i * C_a * (R_i - 1), \quad (\text{Equation 1})$$

where Q_i is the organ's blood flow, C_a is the absolute metabolite concentration in the arterial blood, and R_i is the measured relative concentration ratio between the draining vein of organ i and the arterial blood. Whole-body production or consumption fluxes can then be calculated as

$$F_{\text{production total}} = \sum F_i \text{ for all } F_i > 0, \quad (\text{Equation 2})$$

and

$$F_{\text{consumption total}} = \sum F_i \text{ for all } F_i < 0. \quad (\text{Equation 3})$$

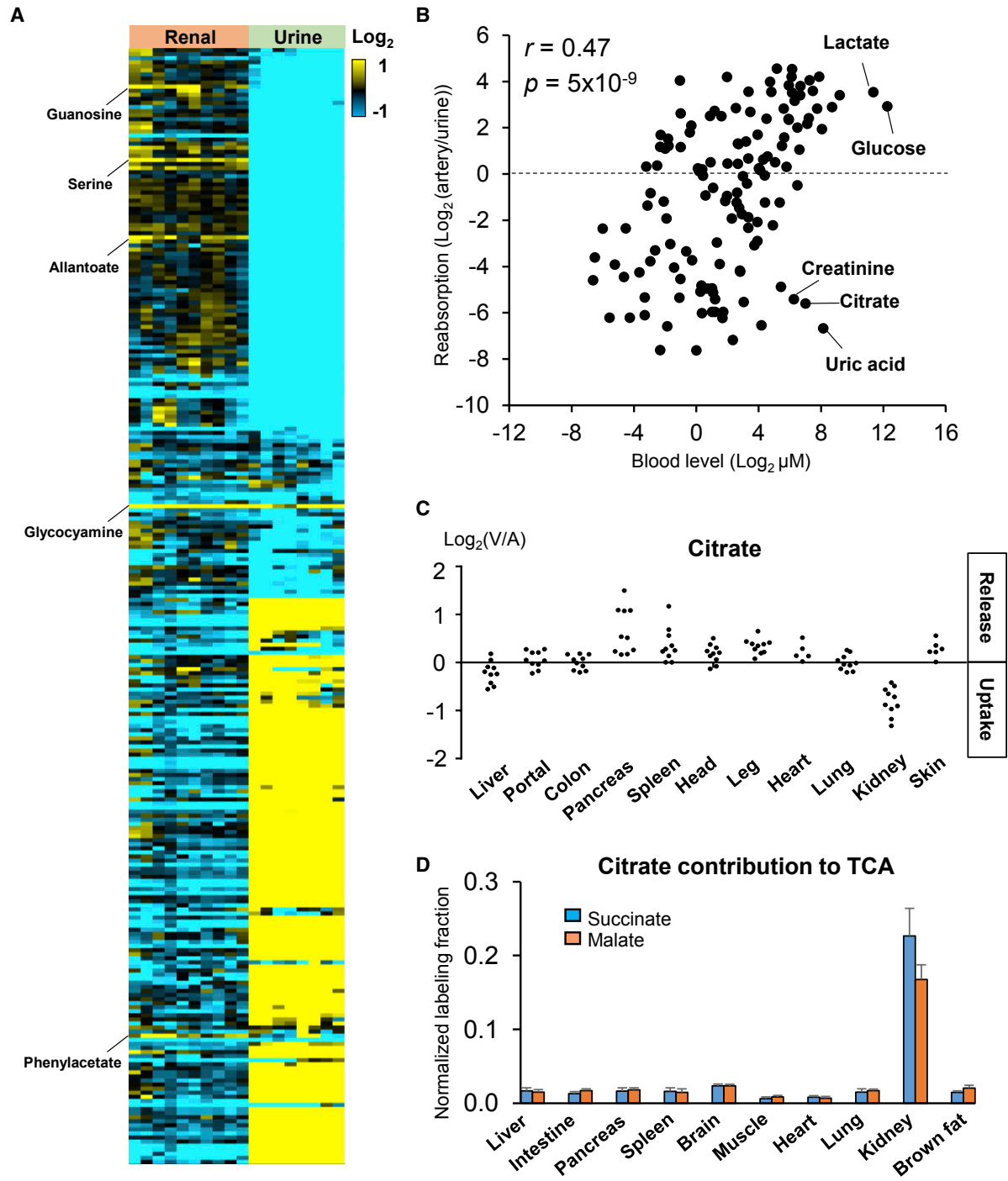


Figure 2. The Kidneys Clear Most Metabolites with Citrate a Kidney-Specific Fuel

(A) Heatmap of metabolite abundance (log_2) in the renal vein relative to the arterial blood (left) or urine relative to arterial blood (right) in pigs. Metabolites produced by the kidneys are highlighted on the left.

(B) Correlation between a metabolite's blood concentration and reabsorption efficiency.

(C) AV difference of citrate across the indicated organs in pigs. Each data point reflects an independent venous blood draw.

(D) Citrate contribution to TCA in mice. The kidneys are unique in using citrate as fuel. Mice were intravenously infused with $[\text{U-}^{13}\text{C}]$ -citrate and average TCA intermediate carbon labeling was normalized to blood-citrate labeling. Data are means and error bars are standard errors ($n = 4$ mice).

See also Figure S3.

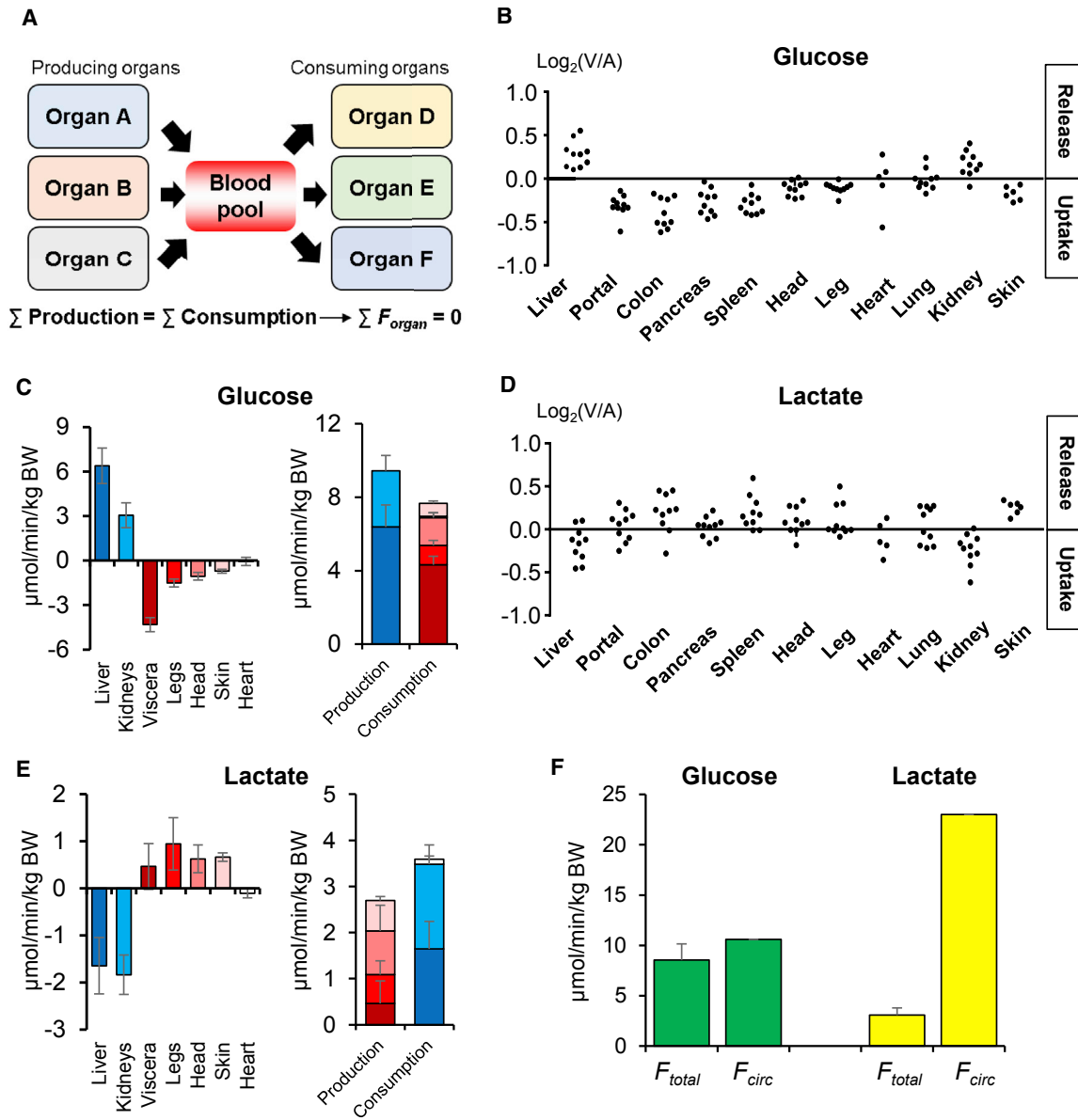


Figure 3. Glucose Production Localizes to the Liver and Kidneys, Whereas Lactate Production Is Distributed throughout the Body

(A) Metabolite production and consumption across all organs must balance to maintain circulating metabolite homeostasis. (B and D) Glucose (B) and lactate (D) show the opposite exchange patterns. Each data point reflects an independent venous blood draw from a pig. (C and E) Calculated production and consumption fluxes of glucose (C) and lactate (E) in the indicated organs in pigs (left sides) and their sum (right sides). (F) Comparison between sum of organ production fluxes (F_{total}) and whole-body production rate (F_{circ}) of glucose and lactate in pigs. Note that F_{total} and F_{circ} are similar for glucose but different for lactate, indicating simultaneous lactate production and consumption within the same organ. Data are means and error bars are standard errors ($n = 5$ pigs, except for isotope tracing, which was performed in a single pig). BW, body weight; viscera, organs feeding into the hepatic portal vein. See also Figure S4.

To maintain circulating concentration homeostasis, production, and consumption fluxes must be balanced. Hence, at steady state

$$\Sigma F_i = 0. \quad (\text{Equation 4})$$

Determination of F_i requires measurement of absolute metabolite AV concentration gradients, not just relative changes. Absolute concentration differences were obtained

by multiplying AV-fold changes by known absolute mammalian metabolite concentrations. Using this approach, we find that consistent with prior literature (Stumvoll et al., 1998), the liver and kidneys produce ~68% and ~32% of circulating glucose (Figures 3B and 3C). Strikingly, in the studied context of fasted anesthetized pig, this glucose is consumed largely by the other visceral organs (~60%) (Figures 3B and 3C). Overall, glucose production and consumption were well-balanced,

supporting this quantitative flux interpretation of AV difference data (Figure 3C).

Lactate is a major secreted product of glucose catabolism, fuel for gluconeogenesis (Stumvoll et al., 1998), and showed the opposite exchange patterns to glucose (Figures 3B and 3D). Lactate and pyruvate share the same carbon skeleton, differing only in redox state. Pyruvate exhibited a similar pattern of organ-specific production and consumption to lactate, with one notable exception: the kidneys that take up lactate but release pyruvate (Figures S4A and S4B). Thus, the kidneys net oxidize lactate to pyruvate, and thereby contribute to circulating redox homeostasis. Consistent with this, chronic kidney disorders are associated with systemic redox imbalance (Pouliant et al., 2016).

Exchange versus Circulatory Flux

Interestingly, although glucose is the most abundant circulating carbohydrate, isotope tracer studies have shown that lactate has higher flux on molar basis (similar on carbon atom basis). This observation is based on ^{13}C - or ^{14}C -isotope tracer studies, dating back decades, which measure the whole-body rate of a metabolite's release from organs into the blood (endogenous production rate) (Coggan, 1999). As the endogenous production rate must balance at steady state with whole body consumption, we term these rates the metabolite's circulatory turnover flux (F_{circ}) (Hui et al., 2017). The high F_{circ} of lactate aligns with recent evidence from ^{13}C -tracing that circulating lactate is a major TCA substrate, with glucose feeding the TCA cycle largely via circulating lactate (Hui et al., 2017; Faubert et al., 2017). Note that ^{13}C -lactate tracing monitors the lactate flux from the bloodstream into tissues that results in its carbon transformation (i.e., conversion to pyruvate and subsequent conversion of pyruvate to acetyl-CoA or oxaloacetate). Exchange of circulating lactate for tissue pyruvate does not alter the carbon skeleton of lactate, and therefore does not contribute to F_{circ} measured with ^{13}C -lactate infusion. We confirmed the higher molar F_{circ} of lactate than glucose by ^{13}C -glucose and ^{13}C -lactate infusion in fasted anesthetized pigs (Figure 3F).

While lactate has higher circulatory turnover flux, glucose shows substantially greater exchange across organs (F_{total}) (Figure 3F). How can these observations be reconciled? Apparently, many organs both produce and consume circulating lactate (i.e., convert circulating glucose to circulating lactate, while also taking in circulating lactate and catabolizing it via the TCA cycle). This reflects the ubiquitous expression of monocarboxylate transporters, which render lactate a nearly universal carbohydrate fuel. Because of the simultaneous lactate consumption and production within an organ (likely by different cells of the organ), lactate exchange between organs is much less than its circulatory turnover flux. In contrast, glucose production and consumption are largely segregated across organs, with the liver and kidneys gluconeogenic and most other organs not producing circulating glucose (Stumvoll et al., 1998). This is evident in the glucose excretion by the liver and kidneys matching closely the whole body endogenous production rate as measured by isotope tracing (F_{circ}) (Figure 3F). Thus, at least in fasted pigs, only the liver and kidneys release substantial glucose into the bloodstream, whereas many organs contain cells that convert glucose to circulating lactate and also cells that take in and metabolize lactate.

Amino Acids

The amino acid with the highest plasma concentration is glutamine, which is an important energy source, gluconeogenic substrate, and nitrogen carrier (Smith, 1990). Consistent with previous AV data in human arm (Ivanisevic et al., 2015), we observed extensive glutamine production by the leg, presumably mainly from skeletal muscle (Figure 4A). The leg's release of glutamine is in part supported by uptake of glutamate. Glutamine release, however, exceeds glutamate consumption by almost an order of magnitude. Thus, the extremities net release not only nitrogen but also the shared glutamine-glutamate carbon skeleton (Figures 4A and S5A). The liver, conversely, consumes glutamine and excretes glutamate (Figure 4A), presumably passing the nitrogen from glutamine into the urea cycle (Smith, 1990). Notably, liver excretion of glutamate slightly exceeds consumption of glutamine, arguing against their shared carbon skeleton being a major net driver of hepatic gluconeogenesis (Stumvoll et al., 1998). In contrast, the other visceral organs and kidneys consume both glutamine and glutamate (Figure 4A), raising the possibility that the glutamine carbon skeleton is an important precursor for renal glucose production.

Serine and glycine, which interconvert via folate metabolism, are another pair of high-flux non-essential amino acids (Ducker and Rabinowitz, 2017). Serine and glycine were produced mainly by the kidneys (Figure 4B), arguing for an important role of renal metabolism in systemic one-carbon homeostasis (Gottlieb and Vousden, 2017).

Essential amino acids (EAAs) cannot be synthesized by mammals and accordingly, in the fasted state, are only produced from protein breakdown. Skeletal muscle is generally believed to be the major amino acid storage organ (Lundholm et al., 1987). In contrast to this paradigm, we observed net uptake of EAAs by the leg, with circulating EAA levels maintained by release from the liver and, surprisingly, also the kidneys (Figure 4C). These findings are consistent with recent data that the liver expands during feeding and contracts during fasting (Sinturel et al., 2017) and suggest that the kidneys may also store protein during feeding and release it during fasting.

Where are these amino acids going? The greatest uptake of both essential and non-essential amino acids occurred in the other visceral organs (Figures 4A–4C and S5B). To examine whether these amino acids were being used for protein synthesis, we carried out ^{13}C -leucine tracer infusion in mice to achieve steady-state labeling of leucine (~15%) in systemic blood (Figure S5C). The liver and kidneys, which showed net release of leucine in pigs (Figure S5B), also incorporated ^{13}C -leucine into proteins (Figure 4D), suggesting that these organs simultaneously synthesize and degrade proteins. However, we observed the fastest protein production in the other visceral organs, which were the greatest amino acid consumers (Figure 4D). Thus, perhaps to support continuous epithelial barrier maintenance and excretory enzyme synthesis, the pancreas and small intestine are persistently anabolic and major resting nutrient consumers.

Collectively, these data contrast with the common assumption that skeletal muscle is the major amino acid depot, releasing EAAs during fasting to supply energy and gluconeogenic precursors to the liver and other organs. Instead, we find that the leg consumes EAAs, while the liver and kidneys produce them.

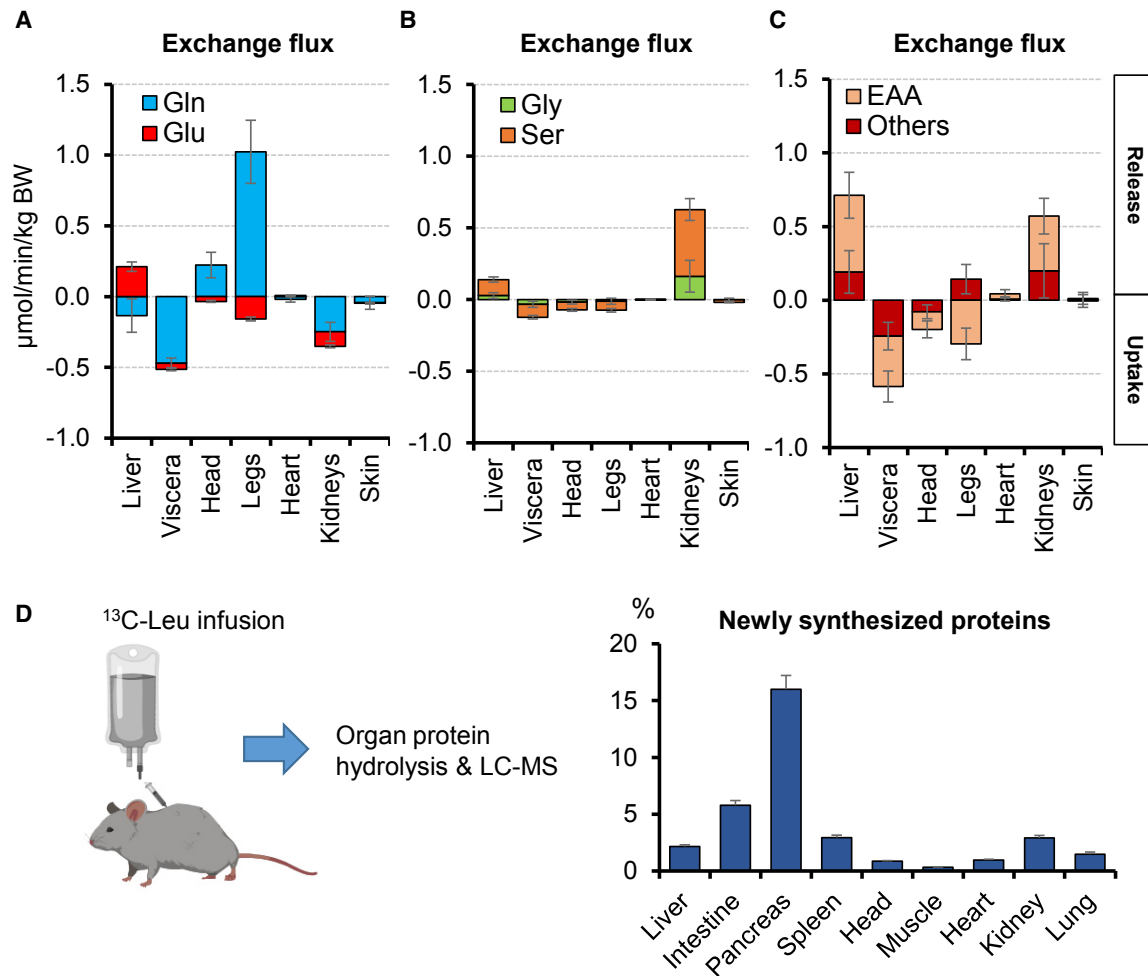


Figure 4. The Liver and Kidneys Release Amino Acids, Which Are Consumed Substantially by the Other Visceral Organs

(A–C) Exchange fluxes of glutamine and glutamate (A), glycine and serine (B), or essential amino acids (EAAs) and other amino acids (Ala, Pro, Arg, and Tyr) (C) in pigs. Data are means and error bars are standard errors (n = 5 pigs).

(D) Newly synthesized proteins in mice. Visceral organs (intestine, pancreas, and spleen) show the most active protein synthesis. Mice were intravenously infused with [U-¹³C]-leucine for 2 h and protein leucine labeling was normalized to blood leucine labeling. Data are means and error bars are standard errors (n = 8 mice). See also [Figure S5](#).

Thus, the present data argue for revisiting the basics of fasting EAA homeostasis.

Short- and Long-Chain Fatty Acids

In addition to being the greatest consumer of glucose and amino acids, the intestines produce a unique set of metabolites from gut microbiota ([Figure 1G](#)). The most abundant ones are short-chain fatty acids (SCFAs) ([Wu et al., 2016](#)). Propionate and butyrate are high in the portal and colonic veins but low in the hepatic vein, confirming their synthesis by microbiota in the gut and efficient clearance by the liver ([Figure S6A](#)) ([Chambers et al., 2018](#)). The most abundant SCFA is acetate. In addition to acetate being released by the gut, acetate is also substantially released from the head ([Figure 5A](#)), suggesting that the brain may synthesize acetate. The acetate is then consumed by several organs including the liver, spleen, legs, and kidneys ([Figure 5A](#)). To explore the fate of acetate in these organs, we conducted steady-state ¹³C-acetate tracer infusions in mice (~20% circu-

lating labeling) ([Figure S6B](#)). The data revealed that these consuming organs use acetate as fuel for TCA metabolism and as substrates for ketone body and fatty acid synthesis ([Figure S6C](#)).

In contrast to the SCFAs, non-esterified long-chain fatty acids (LCFAs) are released by most organs, with the notable exceptions of the liver and heart ([Figure 5B](#)). We suggest that this release is mainly from adipose stores, which are spread throughout the body ([Björntorp, 1991](#)). Additionally, hydrolysis of circulating triglycerides by lipoprotein lipase may contribute ([Barrows et al., 2005](#)). Liver is well known to consume LCFAs, both as an energy source and for packaging into lipoproteins ([Barrows et al., 2005](#)). Detailed analysis of LCFA species, however, revealed an unexpected preference for the liver to consume unsaturated (as opposed to saturated) LCFAs ([Figure 5C](#)). Correspondingly, the leg preferentially releases unsaturated LCFAs ([Figure 5D](#)). We also calculated the absolute exchange fluxes of the three most abundant circulating LCFAs (C16:0, C18:1,

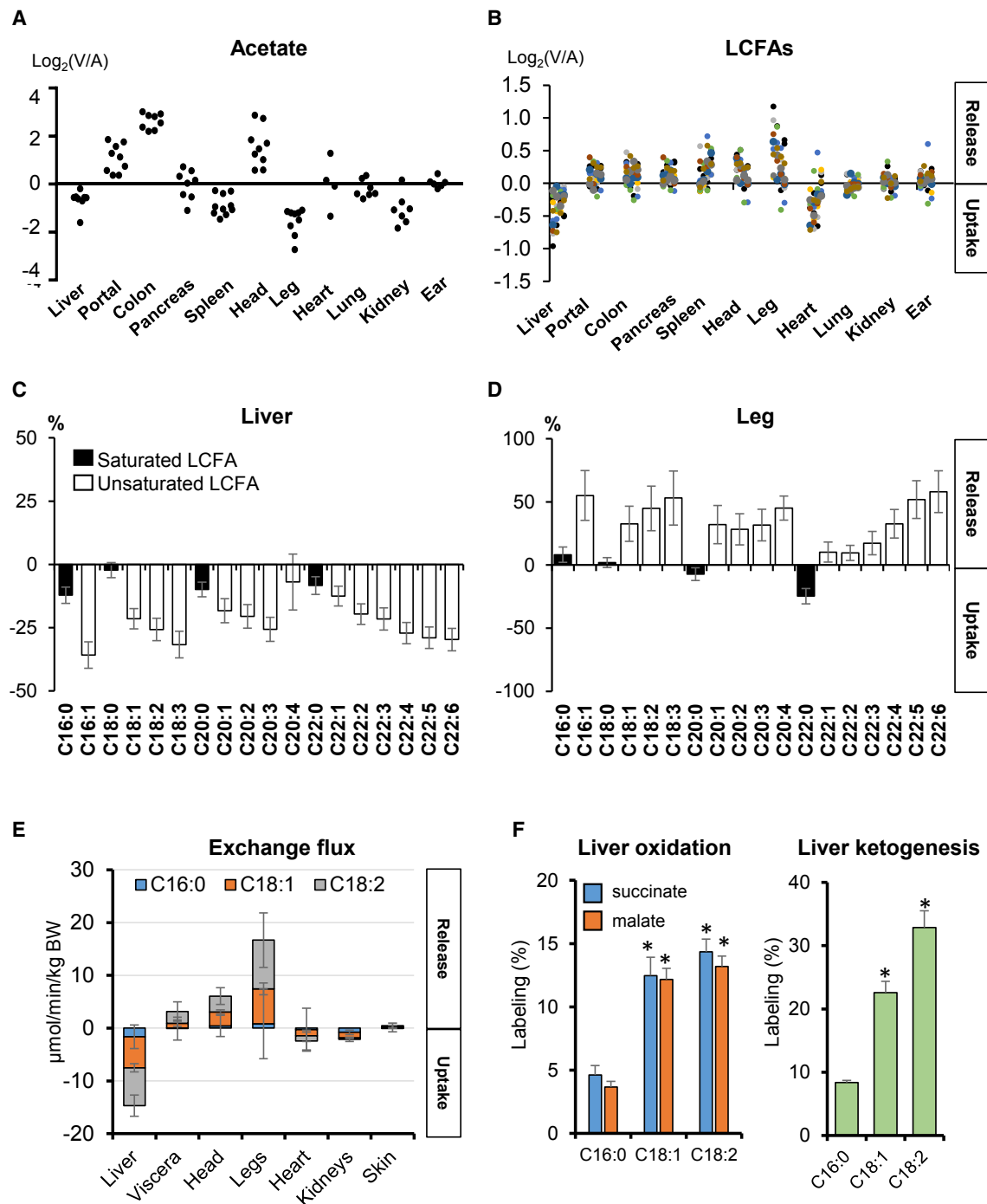


Figure 5. The Liver Preferentially Consumes and Burns Unsaturated as Opposed to Saturated Fatty Acids

(A) Acetate is released from the gut and head. Each data point reflects an independent venous blood draw from a pig.

(B) LCFAs are released from the visceral organs other than liver, head, and leg and consumed by the liver and heart. Each data point reflects the mean AV difference for a specific LCFA in pigs, with different fatty acids shown by different colors.

(C and D) The liver (C) and leg (D) show the opposite preferences in LCFA uptake.

(E) Exchange fluxes of the three most abundant LCFAs in pigs.

(F) The liver preferentially oxidizes unsaturated LCFAs in mice. Mice were intravenously infused with the indicated [$U\text{-}^{13}\text{C}$]-LCFA, and fractions of total labeled carbons in TCA intermediates and 3-hydroxybutyrate in the liver were normalized to blood tracer labeling. Data are means and error bars are standard errors. For (A)–(E), $n = 5$ pigs. For (F), $n = 4, 6,$ and 7 mice for C16:0, C18:1, and C18:2 infusions, respectively. * $p < 0.01$ compared to C16:0 by two-tailed Student's t test.

Viscera, organs feeding into the hepatic portal vein.

See also Figure S6.

and C18:2). The analysis indicated that C18:1 and C18:2 are the high-flux carbon shuttles, being released from the legs and consumed mainly in the liver (Figure 5E). Using ^{13}C -LCFA isotope tracing in mice, we confirmed preferential hepatic oxidation of unsaturated versus saturated LCFAs (Figure 5F), which is consistent with preferential hepatic uptake of unsaturated LCFAs in pigs (Figure 5E). Thus, the existence of double bond(s) in LCFAs plays critical roles in determining uptake and oxidation efficacies by organs, which may be linked to differential effects of saturated versus unsaturated LCFAs on cardiovascular and metabolic disease (Jha et al., 2018).

Conclusions and Future Perspectives

Here, we provide a quantitative atlas of inter-organ metabolite exchange. In addition to confirming classical organ-specific metabolic functions like renal and hepatic glucose production, our systems-level analysis identified unexpected ones. These include previously unknown sources and sinks for hundreds of metabolites, with 739 total observations of significant organ-specific metabolite production or consumption (Table S1; Data 1). Many of these observations involve less studied metabolites and provide a foundational resource for understanding their physiological role and homeostatic mechanisms. Even for glucose itself, we obtained new insights, most notably extensive glucose consumption by the visceral organs, highlighting an underappreciated role of the intestine, which can be gluconeogenic in some contexts (Mithieux, 2005; Jang et al., 2018), in clearing fasting blood glucose.

Looking forward, a key challenge is quantitative integration of AV difference and isotope tracing data. These two data types are complementary, as AV difference data measure *net* metabolic activity (production minus consumption) whereas isotope tracing measures *gross* metabolic activity (total production or consumption). When these align, conceptual understanding is straightforward, e.g., we find that the liver preferentially consumes and burns unsaturated versus saturated fatty acids. Differences, however, can also be highly informative. For example, although we observe a propensity for net fatty acid release from the leg, isotope tracing showed that fatty acids are a major muscle fuel (Guo and Jensen, 1998). This reflects the physiology of the body part, with adipose and muscle in proximity and playing different metabolic roles in fat management.

In sum, our data provide a comprehensive resource of net organ-specific production and consumption fluxes. These are critical inputs for ongoing efforts to quantitatively model mammalian metabolism (Ahn et al., 2017; Noronha et al., 2017; Thiele et al., 2013; Shlomi et al., 2008). As such, they are a key step toward understanding the homeostasis of the circulating metabolome.

LIMITATIONS OF STUDY

The AV difference measurements reported here are based on fasted, anesthetized pigs. They reflect the integrative function of the cell types and organs drained by the sampled vein (for example, internal jugular vein drains not only the brain but also the face). Metabolism can change dramatically between feeding and fasting. Some anticipated metabolic changes in the fed state include absorption of copious glucose and amino acids from the

intestinal lumen, altered access of the microbiome to dietary nutrients (Gentile and Weir, 2018), and insulin-induced muscle-glucose catabolism and suppression of adipose lipolysis (Saltiel, 2016). The present measurements may also have been impacted by anesthesia, which alters brain glucose usage (Bascañana et al., 2019) and may also impact skeletal muscle metabolism due to deficient afferent CNS inputs and associated lack of muscle contraction. Anesthesia facilitated blood sampling, especially from deeply buried veins like the hepatic, splenic, and colonic. It is not, however, required for sampling from more accessible sites and AV studies have previously been conducted in awake humans and dogs (Davis et al., 1985; Felig and Wahren, 1971; Felig et al., 1969; Moore et al., 1998; Wahren et al., 1972). Studies sampling from a more limited set of veins in awake mammals are a valuable complement to the present research and can potentially be coupled to physiological manipulations of metabolic demand (e.g., exercise and thinking). Comprehensive AV measurements in fed animals are a key future objective.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- CONTACT FOR REAGENT AND RESOURCE SHARING
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
 - Pig Experiments
 - Mouse Experiments
- METHOD DETAILS
 - Metabolite Extraction
 - Metabolite Measurement by LC-MS
 - SCFA Measurement
 - Protein Labeling Measurement
- QUANTIFICATION AND STATISTICAL ANALYSIS
- DATA AND CODE AVAILABILITY

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at <https://doi.org/10.1016/j.cmet.2019.06.002>.

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AUTHOR CONTRIBUTIONS

C.J., R.J.M., and J.D.R. designed the study. C.J. performed most experiments. S.H. contributed to isotope tracing studies in pigs and mice, modeling, and data analysis. X.Z. contributed to isotope tracing studies in mice, developed the analytical method for SCFA, and collected the SCFA data. A.J.C. contributed to pig experiments and sample preparation. J.R. contributed to the flux analysis. L.W. and L.C. contributed to chemical formula assignment of putative metabolites. C.F. contributed the ideas on kidney cancer. H.Y.H., A.I., Y.S., K.O., C.V., L.K., and G.A.Z. performed pig surgery and blood collection. J.H.G. and R.C.G. provided surgical expertise and intellectual guidance. C.J. and J.D.R. wrote the manuscript. All authors discussed the results and commented on the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, Peptides, and Recombinant Proteins		
L-LACTATE (U-13C, 98%)	Cambridge Isotope Laboratories	Cat#CLM-1579-PK
D-GLUCOSE (U-13C, 99%)	Cambridge Isotope Laboratories	Cat#CLM-1396-PK
CITRATE (U-13C, 98%)	Cambridge Isotope Laboratories	Cat#CLM-9021-PK
L-MALATE (U-13C, 99%)	Cambridge Isotope Laboratories	Cat#CLM-8065-PK
SUCCINATE (U-13C, 99%)	Sigma	Cat#491985
ACETATE (U-13C, 99%)	Cambridge Isotope Laboratories	Cat#CLM-440-PK
OLEATE (U-13C, 98%)	Cambridge Isotope Laboratories	Cat#CLM-8763-PK
LINOLEATE (U-13C, 98%)	Cambridge Isotope Laboratories	Cat#CLM-8735-PK
PALMITATE (U-13C, 98%)	Cambridge Isotope Laboratories	Cat#CLM-3943-PK
L-LEUCINE (U-13C, 99%)	Cambridge Isotope Laboratories	Cat#CLM-2262-PK
Fatty acid free BSA	Sigma	Cat#A6003
XBridge BEH Amide XP column	Waters	Cat#176002889
Acquity UPLC BEH C18 column	Waters	Cat#186003719
EDC	Sigma	Cat#03449
3-Nitrophenylhydrazine	Sigma	Cat#N21804
Pyridine	Sigma	Cat#270970
beta-mercaptoethanol	Sigma	Cat#M6250
Experimental Models: Organisms/Strains		
Mouse: C57BL/6	Charles River Laboratories	Cat#027
Pig: Yorkshire	Meck Swine LLC	N/A
Software and Algorithms		
MAVEN software	http://genomics-pubs.princeton.edu/mzroll/index.php	Princeton University

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Joshua Rabinowitz (joshr@princeton.edu).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Pig Experiments

Pig studies followed protocols approved by the University of Pennsylvania Institutional Animal Care and Use Committee. Five-month old male Yorkshire pigs were fed at 4 PM and then overnight fasted. At 7 AM in the next morning, anesthesia was initiated with a ketamine IM injection (15~35 mg/kg), followed by intubation and maintenance of anesthesia with inhaled isoflurane at 0.5~5%. Infusion of [U-¹³C]-glucose or [U-¹³C]-lactate was performed via the external jugular vein catheter. Blood was collected from the carotid artery every 10 min during the infusion. For the glucose, after a bolus of 0.47 mL/kg of 200 mM solution, infusion was performed with 0.04 mL/min/kg of 26 mM solution for 110 min to achieve steady-state blood labeling (9%). For the lactate infusion, infusion was performed with 0.035 mL/min/kg of 82 mM solution for 110 min to achieve steady-state blood labeling (11%). Arterial and venous access was obtained to enable fluid infusion (NaCl 4 - 10 mcg/kg/min), blood pressure monitoring, pharmacological control of blood pressure (0.2 - 2.0 mcg/kg/min dobutamine and 0.1 - 5.0 mcg/kg/min phenylephrine, as required), and blood sampling. The primary initiative venous access was via ultrasound-guided percutaneous puncture of the external jugular vein. Arterial access was through a neck to the carotid artery. The surgical site was blocked with Bupivacaine SQ just after the first incision. The subcutaneous tissues and muscle layers were carefully dissected, with hemostasis achieved with electrocautery. For blood sampling from the hepatic and the portal vein, a catheter was placed on each vein. Blood from inferior vena cava, internal jugular vein, femoral vein, renal vein, splenic vein, inferior pancreatic vein, inferior mesenteric vein, lateral auricular vein, and coronary sinus were obtained by drawing with a 27G needle connected to 1 mL syringe. Blood was drawn from each vessel twice at 1 - 2 min intervals, except for the coronary sinus (only a single draw). For urine, a catheter was implanted into the bladder, residual urine was drained, and a sterile 50 mL falcon

tube was connected to the catheter to collect fresh urine. After completion of blood sampling, the animal was euthanized. Blood samples were placed at room temperature for 10 - 20 min, and centrifuged at 1,000 x g for 10 min to obtain serum.

Mouse Experiments

Mouse studies followed protocols approved by the Princeton University Institutional Animal Care and Use Committee. Infusion was performed on single-housed 10 - 14 week old male C57BL/6 mice with a catheter surgically implanted on the right jugular vein (Charles River). ^{13}C -organic acid infusion solutions were prepared in saline with 20 mM $[\text{U-}^{13}\text{C}]$ -sodium citrate, 5mM $[\text{U-}^{13}\text{C}]$ -sodium malate, 10 mM $[\text{U-}^{13}\text{C}]$ -sodium succinate, or 200 mM $[\text{U-}^{13}\text{C}]$ -sodium acetate. Infusion was performed with 0.1 $\mu\text{L/g/min}$ for 3 hours to achieve steady-state blood labeling (10~15%) without perturbation of circulating blood levels. For ^{13}C -LCFA infusions, $[\text{U-}^{13}\text{C}]$ -LCFA was first dissolved in 70°C saline at 16 mM and slowly mixed with 37°C saline containing 4 mM fatty acid-free BSA at 1:1 v/v ratio to conjugate LCFA to albumin at 4:1 ratio. Final infusion solutions were 8 mM for $[\text{U-}^{13}\text{C}]$ -sodium oleate or $[\text{U-}^{13}\text{C}]$ -sodium linoelate and 4 mM for $[\text{U-}^{13}\text{C}]$ -sodium palmitate. Infusion was performed with 0.5 $\mu\text{L/g/min}$ for 3 hours to achieve steady-state blood labeling (8~10%) without perturbation of circulating blood levels. The mouse infusion setup (Instech Laboratories, Plymouth Meeting, PA) included a tether and swivel system so that the animal has free movement in the cage. On the day of the experiment, mice were transferred to new cages without food at 10 AM for fasting. At 3 PM, infusion was started. Blood (~10 μL) was collected by tail bleeding in blood collection tubes (Sarstedt 16.443.100), placed on ice for 20 min, and centrifuged at 16,000 x g for 10 min at 4°C to obtain serum. For tissue harvest, mice were euthanized via cervical dislocation and tissues were quickly dissected and snap frozen in liquid nitrogen with a pre-cooled Wollenberger clamp. Serum and tissue samples were kept at -80°C until LC-MS analysis. All mouse experiments were performed with 2 or 3 mice per day, with results validated on multiple days using mice from different cages.

METHOD DETAILS

Metabolite Extraction

Serum (5 μL) was mixed with 150 μL -20°C 40:40:20 methanol:acetonitrile:water (extraction solvent), vortexed, and immediately centrifuged at 16,000 x g for 10 min at 4°C. The supernatant was collected for LC-MS analysis. To extract metabolites from tissue samples, frozen tissue samples were ground at liquid nitrogen temperature with a Cryomill (Retsch, Newtown, PA). The resulting tissue powder (~20 mg) was weighed and then extracted by adding -20°C extraction solvent (as above), vortexed, and immediately centrifuged at 16,000 x g for 10 min at 4°C. The volume of the extraction solution (μL) was 40x the weight of tissue (mg) to make an extract of 25 mg tissue per mL solvent. The supernatant was collected for LC-MS analysis.

Metabolite Measurement by LC-MS

A quadrupole-orbitrap mass spectrometer (Q Exactive, Thermo Fisher Scientific, San Jose, CA) operating in negative or positive ion mode was coupled to hydrophilic interaction chromatography via electrospray ionization and used to scan from m/z 70 to 1000 at 1 Hz and 75,000 resolution. LC separation was on a XBridge BEH Amide column (2.1 mm x 150 mm, 2.5 μm particle size, 130 Å pore size; Waters, Milford, MA) using a gradient of solvent A (20 mM ammonium acetate, 20 mM ammonium hydroxide in 95:5 water: acetonitrile, pH 9.45) and solvent B (acetonitrile). Flow rate was 150 $\mu\text{L/min}$. The LC gradient was: 0 min, 85% B; 2 min, 85% B; 3 min, 80% B; 5 min, 80% B; 6 min, 75% B; 7 min, 75% B; 8 min, 70% B; 9 min, 70% B; 10 min, 50% B; 12 min, 50% B; 13 min, 25% B; 16 min, 25% B; 18 min, 0% B; 23 min, 0% B; 24 min, 85% B; 30 min, 85% B. Autosampler temperature was 5°C, and injection volume was 3 μL . Data were analyzed using the MAVEN software. For tracer experiments, isotope labeling was corrected for natural ^{13}C abundance (Su et al., 2017).

SCFA Measurement

SCFAs were derivatized and measured by LC-MS. The derivatizing reagent was 12 mM EDC, 15 mM 3-Nitrophenylhydrazine and pyridine (2% v/v) in methanol. Reaction was stopped with quenching reagent consisting of 0.5 mM beta-mercaptoethanol and 0.1% formic acid in water. Serum (5 μL) was mixed with derivatizing reagent (100 μL) and incubated for 1 hour at 4°C. Then, the samples were centrifuged at 16,000 x g for 10 min at 4°C, and 20 μL of supernatant was mixed with 200 μL of the quenching reagent. After centrifugation at 16,000 x g for 10 min at 4°C, supernatants were collected for LC-MS analysis. A quadrupole-time of flight mass spectrometer (Q-TOF, Agilent, Santa Clara, CA) operating in negative ion mode was coupled to C18 chromatography via electrospray ionization and used to scan from m/z 100 to 300 at 1 Hz and 15,000 resolution. LC separation was on an Acquity UPLC BEH C18 column (2.1 mm x 100 mm, 1.75 μm particle size, 130 Å pore size; Waters, Milford, MA) using a gradient of solvent A (0.01% formic acid in water) and solvent B (0.01% formic acid in isopropanol). Flow rate was 400 $\mu\text{L/min}$, except that from 6 min to 8 min flow rate was increased to 700 $\mu\text{L/min}$. The LC gradient was: 0 min, 10% B; 2 min, 15% B; 5 min, 25% B; 6 min, 100% B; 8 min, 100% B; 8.6 min, 10% B; 10.5 min, 10% B. Autosampler temperature was 5°C, and injection volume was 10 μL . Ion masses for derivatized formate, acetate, propionate, and butyrate were 180, 194, 208, and 222, respectively.

Protein Labeling Measurement

Infusion solution was prepared in saline with $[\text{U-}^{13}\text{C}]$ -leucine, unlabeled valine and isoleucine (45, 37.5, and 26.25 mM, respectively), as previously described (Neinast et al., 2019). Infusion was performed for 2 hours at the rate of 0.0836 $\mu\text{L/g/min}$. Tissue proteins were

isolated by chloroform-methanol extraction. Protein pellets were washed with methanol twice to remove residual amino acids. Then, the protein samples were hydrolyzed with 6 M HCl at 120°C overnight. After completely drying the HCl with blowing nitrogen gas, the samples were dissolved with 80% methanol in water, centrifuged at 16,000 x g for 10 min at 4°C, and supernatants were collected for LC-MS analysis.

QUANTIFICATION AND STATISTICAL ANALYSIS

The absolute metabolite concentrations in the blood were taken from the Human Metabolome Database, using the average of healthy male data. Regional blood flow was from (Iaizzo, 2005). A two-tailed unpaired Student's t-test was used to calculate p-values. Metabolomics data were corrected for multiple comparisons, based on the number of measured metabolites, via the Benjamini-Hochberg method, with a false-discover rate (FDR) cut off of 0.05 used to determine statistical significance.

DATA AND CODE AVAILABILITY

All the pig A/V data are provided in Data 3. MAVEN software for LC-MS data analysis is available online at <http://genomics-pubs.princeton.edu/mzroll/index.php>.