Supplementary Materials for

Comprehensive quantification of fuel use by the failing and nonfailing human heart

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Materials and Methods

Study Population and Sample Collection

All patients were undergoing elective radiofrequency catheter ablation for treatment of atrial fibrillation or ventricular tachycardia. The study protocol was approved by the Institutional Review Board of the Hospital of the University of Pennsylvania (Federalwide Assurance #00004028, Board #1 Protocol #827174). All study participants provided written informed consent. All patients were >18 years of age and non-pregnant. All patients had fasted overnight and were under general anesthesia with propofol, remifentanil, etomidate, and succinylcholine. Following induction of general anesthesia, a 20-gauge catheter was inserted into the radial artery to continuously monitor blood pressure. Hemostatic sheaths were inserted into the femoral veins. One of these sheaths was exchanged for a long SL0^{TM} sheath (Abbott, Chicago, IL) which was advanced over a diagnostic catheter into the coronary sinus. Within 60 minutes of induction of anesthesia, samples were simultaneously drawn from the radial artery, femoral vein, and coronary sinus. All blood samples were drawn prior to initiation of ablation. Mean right and left atrial chamber pressures were measured immediately prior to and following transseptal puncture and are reported as the average of the two measurements recorded by the two transseptal sheaths. Echocardiographic findings are reported from the most recent echocardiogram done prior to procedure. Blood samples were collected in lithium-heparin treated vacutainers and placed immediately on ice. Plasma was separated by centrifugation at 3,000g at 4°C for 10 minutes, and all samples were stored at -80°C until analysis. Blood oxygen content was measured in arterial, coronary sinus, and femoral venous samples of 17 patients at the time of sample collection using an ABL90 FlexPlus blood gas analyzer (Radiometer, Brea, CA).

Metabolite extraction

Plasma (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. [U-^{13}C]-valine was spiked in the extraction solvent as an internal standard to account for instrument variability. Quantification of hypoxanthine, uric acid, and 3-hydroxybutyrate was performed by spiking known concentrations of [U-^{13}C]-hypoxanthine and [U-^{13}C]-3-hydroxybutyrate in individual patient samples or by fitting to a standard curve of uric acid.

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 140,000 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 µL/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. Metabolites were confirmed by exact mass and retention time match to authenticated standards.

Measurement of acetate
Acetate was 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 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, 1.7 5 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 µL/min, except that from 6 min to 8 min flow rate was increased to 700 µL/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 acetate was 208.

Measurement of glucose, lactate, glutamine, glutamate
Glutamine, glutamate, glucose, and lactate concentrations were measured using a YSI 2900 Series Biochemistry Analyzer (YSI Inc./Xylem Inc., Yellow Springs, OH). System buffer (YSI 2357), standards (YSI 2776, YSI 1531, YSI 1530, YSI 2736, YSI 2755, YSI 2737, YSI 2756), membranes (YSI 2365, YSI 2329, YSI 2735, YSI 2754) were purchased from YSI Inc./Xylem Inc. (Yellow Springs, OH). For glucose/lactate measurement, plasma samples were diluted 1:2 in water for a final sample volume of 25 µl. For glutamine/glutamate measurement, 20 ul of plasma was directly measured. Reported values are an average of two readings per patient sample.

Insulin measurement
Insulin concentrations were determined in triplicate using the commercially available Insulin Human ELISA kit (Crystal Chem cat number 90095, lot number 911554; Elk Grove Village, IL).

Calculations and Statistical Analysis
Peak intensities for all 277 circulating metabolites were normalized to the median arterio-venous ratio for each patient's paired arteriovenous comparison. Metabolomics data were corrected according to the Benjamini-Hochberg method using a false-discovery rate cutoff of 0.05 to determine statistical significance. For metabolites where concentration was directly measured (listed in Table S4), the patient-specific concentration of each metabolite was used for subsequent calculations. For all other metabolites, the absolute concentrations were taken from the Human Metabolome Database. For flux calculations that required coronary blood flow, a value of ~0.8 ml/min/g cardiac tissue was assumed.

Calculation of amino acid clearance
Cardiac amino acid release from proteolysis was calculated using the net amino acid A-V nitrogen balance across the heart and the average overall amino acid composition of muscle protein (*14–*

17, *40*). For all calculations, each patient's measured amino acid concentration was used for those amino acids listed in Table S5. Published concentrations were used for methionine, glycine, cysteine, and aspartate.

The net A-V nitrogen balance from amino acid uptake and release was calculated for each patient according to:

$$\Delta N_{A.A.} = \sum([N_{A.A.}]_{C.S.} - [N_{A.A.}]_{Art})$$

where $N_{A.A.}$ represents total amino acid nitrogen concentration. For a given amino acid i ($A.A._i$), the net uptake or release of $A.A._i$ is dictated by the net liberation of $A.A._i$ from proteolysis and net metabolic combustion of $A.A._i$:

$$\Delta[A.A._i]_{AV} = [A.A._{i.}]_{C.S.} - [A.A._i]_{Art}$$

$$\Delta[A.A._i]_{AV} = [net\ liberation\ of A.A._{i.}\ from\ protein] - [net\ combustion\ of\ A.A._{i.}]$$

The net liberation of $A.A._i$ from protein was calculated using the net amino acid A-V nitrogen balance and the typical composition of muscle protein using the following:

$$\text{net liberation of } A.A._i \text{ from protein} = a_i\left(\frac{\Delta N_{A.A.}}{k}\right)$$

where a_i is the abundance of amino acid i in protein and k is the average weighted nitrogen content per amino acid in myocardial protein. A value of $k = 1.38$ was used here. Thus, net myocardial combustion of $A.A._i$:

$$\text{net combustion of } A.A._i = a_i\left(\frac{\Delta N_{A.A.}}{k}\right) - \Delta[A.A._i]_{AV}$$

Several corrections were performed to account for the interconversion of amino acids to other combusted fuels (e.g. alanine, lactate) or the release of modified intermediates (e.g. arginine, n-acetyl-arginine). All of the following corrections were handled for each patient individually using his or her A/V ratios and measured plasma concentrations (where available) or published values. For all scenarios listed in the table below, the "net contribution to combustion" was used to calculate net contribution to myocardial O_2 consumption and ATP synthesis. Appropriate consideration was made in cases where the number of carbons differs among metabolites (e.g. cystine $C_6H_{12}N_2O_4S_2$ vs. cysteine $C_3H_7NO_2S$). In the table below, metabolites are listed under "uptake" or "release" based on whether each metabolite is typically taken up or released by the heart; however, the net combustion of these metabolites in each patient was ultimately handled on a case-by-case basis.

Special Case	Contribution from metabolite uptake	Contribution from proteolysis	Deduction from metabolite release	Net contribution to combustion
Lactate, Alanine	Δ $Lactate_{A-V}$	$\Delta Ala_{protein}$	ΔAla_{A-V}	Net lactate combustion = $\Delta Lactate_{A-V} + \Delta Ala_{protein} - \Delta Ala_{A-V}$
Glutamate, Glutamine	Δ Glu_{A-V}	$\Delta Gln_{protein}$ $\Delta Glu_{protein}$	ΔGln_{A-V}	Net Glu combustion = ΔGlu_{A-V} + $\Delta Gln_{protein}$ + $\Delta Glu_{protein}$ - ΔGln_{A-V}
Glycine, Serine, 2-hydroxyhippuric acid, N-acetyl-glycine		$\Delta Gly_{protein}$ $\Delta Ser_{protein}$	ΔGly_{A-V} ΔSer_{A-V} Δ2-hydroxyhippuric $acid_{A-V}$ ΔN-acetyl-$glycine_{A-V}$	Net Ser combustion = ΔSer_{A-V} + $\Delta Gly_{protein}$ +$\Delta Ser_{protein}$ -ΔGly_{A-V} -Δ2-hydroxyhippuric $acid_{A-V}$ -ΔN-acetyl-$glycine_{A-V}$
Proline, Hydroxyproline		$\Delta Pro_{protein}$	ΔPro_{A-V} $\Delta hydroxyproline_{A-V}$	Net Pro combustion = $\Delta Pro_{protein}$ -ΔPro_{A-V} -$\Delta hydroxyproline_{A-V}$
Arginine, N-acetyl-arginine		$\Delta Arg_{protein}$	ΔArg_{A-V} ΔN-acetyl-$arg._{A-V}$	Net Arg combustion = $\Delta Arg_{protein}$ -ΔArg_{A-V} -ΔN-acetyl-arg_{A-V}
Methionine, Homocysteine		$\Delta Met_{protein}$	ΔMet_{A-V} $\Delta homocysteine_{A-V}$	Net Met combustion = $\Delta Met_{protein}$ -ΔMet_{A-V} -$\Delta homocysteine_{A-V}$
Cystine, Methylcysteine		$\Delta Cys_{protein}$	ΔCys_{A-V} $\Delta methylcysteine_{A-V}$	Net Cys combustion = $\Delta Cys_{protein}$ - ΔCys_{A-V} -$\Delta methylcysteine_{A-V}$

Calculation of oxygen requirements and ATP yield

Predicted oxygen requirements for substrate catabolism were based on theoretical full oxidation of metabolites that were consumed by the heart:

$$C_aH_bO_cN_d + xO_2 \rightarrow CO_2 + H_2O$$

For amino acids, the concentration of metabolite consumed was calculated based on the net combustion of each amino acid as calculated above. In the case of alanine and glutamine, both of which are net released after adjustment for catabolism of amino acid from protein, the equivalent

oxygen requirement to fully combust the released quantity of amino acid was subtracted from lactate and glutamate, respectively. Amino acids that cannot be catabolized by the heart (Phe, His, Tyr) (*41*) were not included in calculation of predicted oxygen requirement for full catabolism of consumed substrates (**Fig. 3A**).

Calculation of substrate contribution to cardiac ATP production was performed using published values of ATP yields from substrate oxidation (*42–44*). Values for amino acid combustion (µM amino acid) were the same as those used to calculate oxygen requirements.

ATP yield from unmeasured LpFA and FFA (**Fig. 3B**, gray) was calculated assuming that O_2 consumption due to unmeasured metabolites (**Fig. 3A**, gray) was due to complete oxidation of FAs in proportion to their measured concentrations in arterial plasma.

Adjustment of metabolite extraction

To account for presumed lower myocardial blood flow in patients with reduced EF, the extracted amount of acetate, 3-hydroxybutyrate and glutamate in patients with rEF was scaled in **Fig. 4B** and **Fig. S10** according to:

$$(C_{CS}\text{-}C_A)_{scaled} = k(C_{CS}\text{-}C_A)_{raw}$$

where:

$$k = \frac{1-y}{1-z}$$

$$y = \text{average}[(\frac{C_{CS}}{C_A})_{acetate,\ pEF}]$$
$$z = \text{average}\ [(\frac{C_{CS}}{C_A})_{acetate,\ rEF}]$$

Fig. S1.

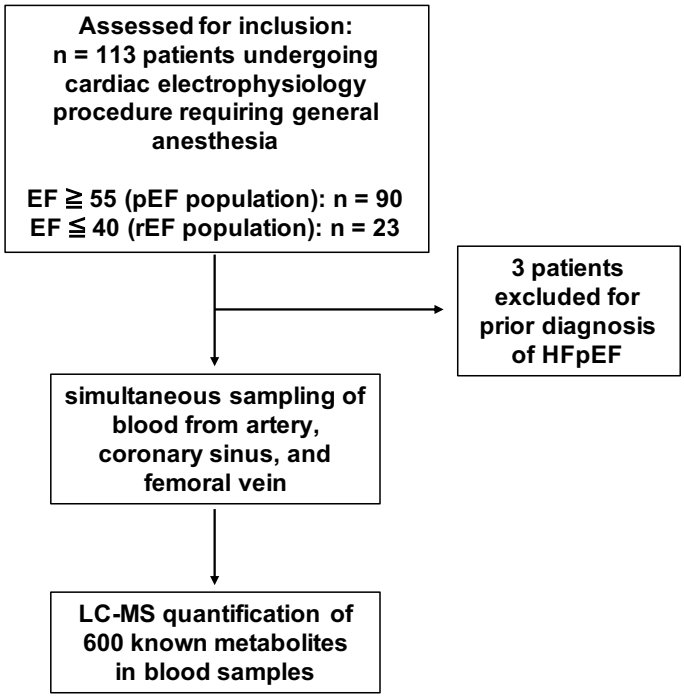

Fig. S1. Study design.

Fig. S2.

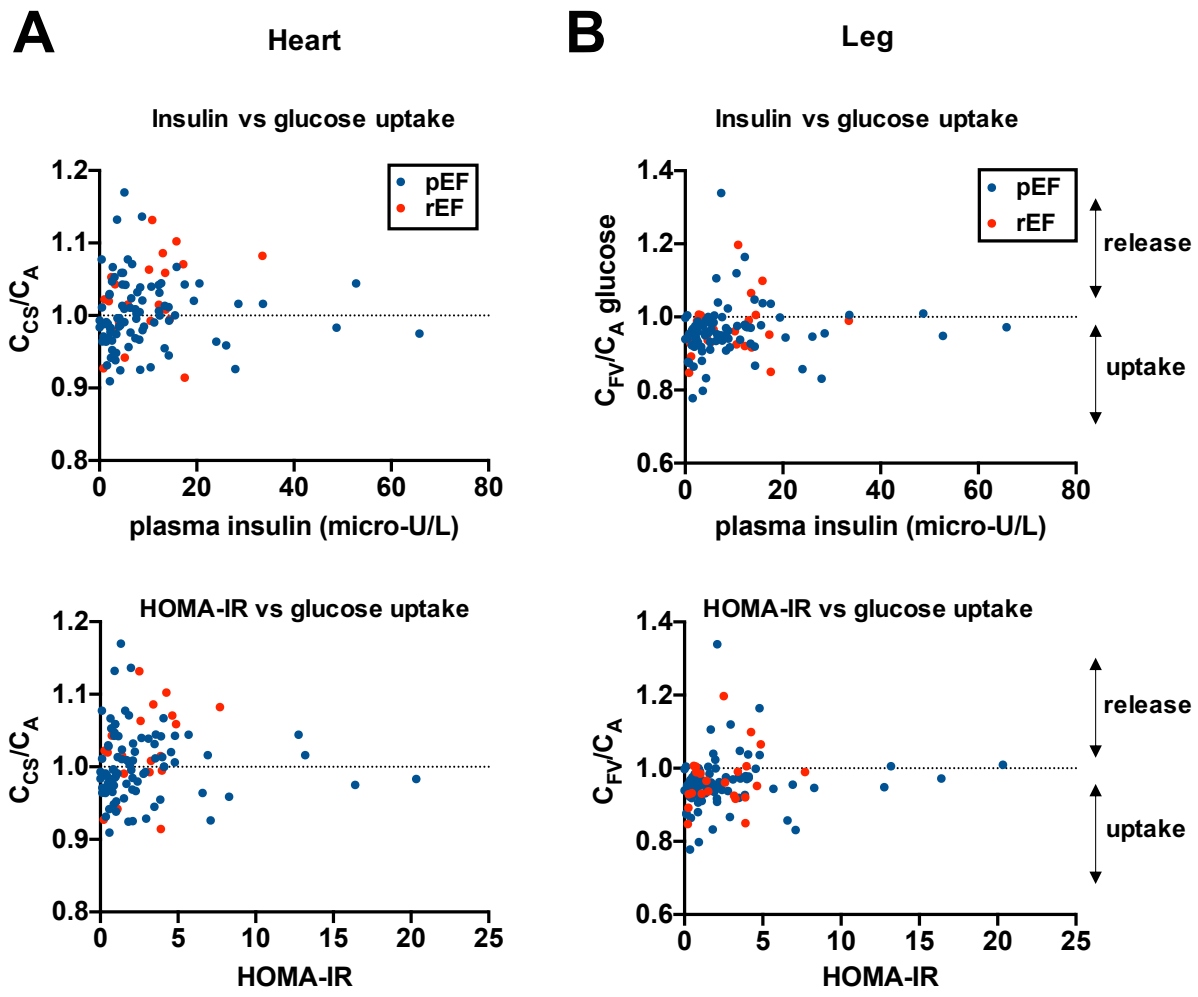

Fig. S2. Relationship between plasma insulin, HOMA-IR and glucose uptake by the heart and leg in pEF and rEF. Plasma insulin was measured from arterial plasma; C_{CS}/C_A **(A)** or C_{FV}/C_A **(B)** > 1 corresponds to glucose release; C_{CS}/C_A or C_{FV}/C_A < 1 corresponds to glucose uptake.

Fig. S3.

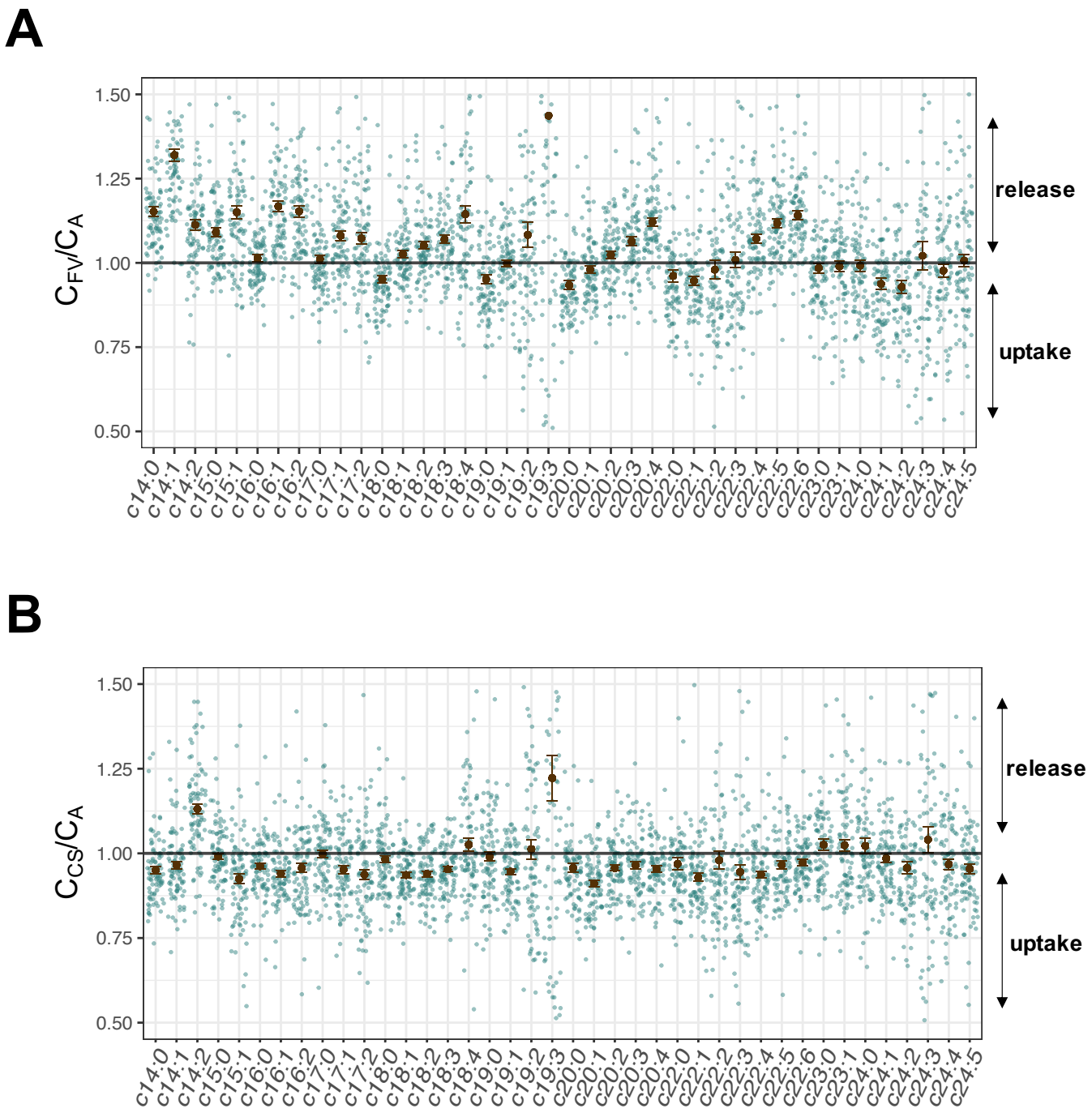

Fig. S3. Free fatty acid balance across the leg **(A)** and heart **(B)** depicted as C_{FV}/C_A or C_{CS}/C_A, respectively; C_{CS}/C_A or $C_{FV}/C_A > 1$ corresponds to release; C_{CS}/C_A or $C_{FV}/C_A < 1$ corresponds to uptake.

Fig. S4.

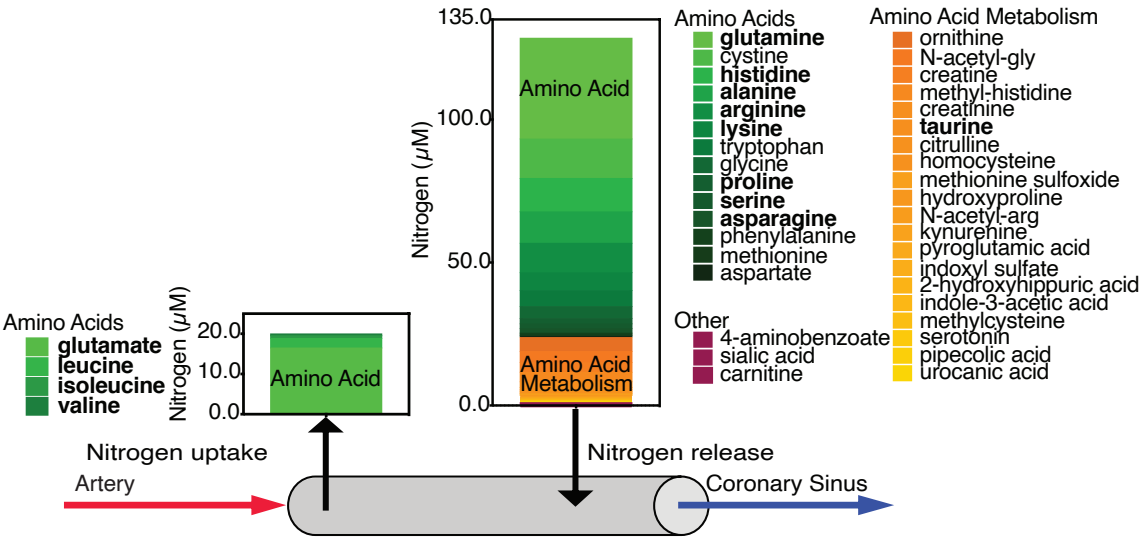

Fig. S4. Total nitrogen release by the heart

(A) Total average nitrogen (N) uptake or release (μM N) by the heart in the form of amino acids and amino acid metabolites. Metabolites in bold were measured directly in plasma. All N-containing amino acids, modified amino acids, and intermediates in amino acid catabolism with average $|C_{CS} - C_A| > 0.1$ uM are included.

Fig. S5.

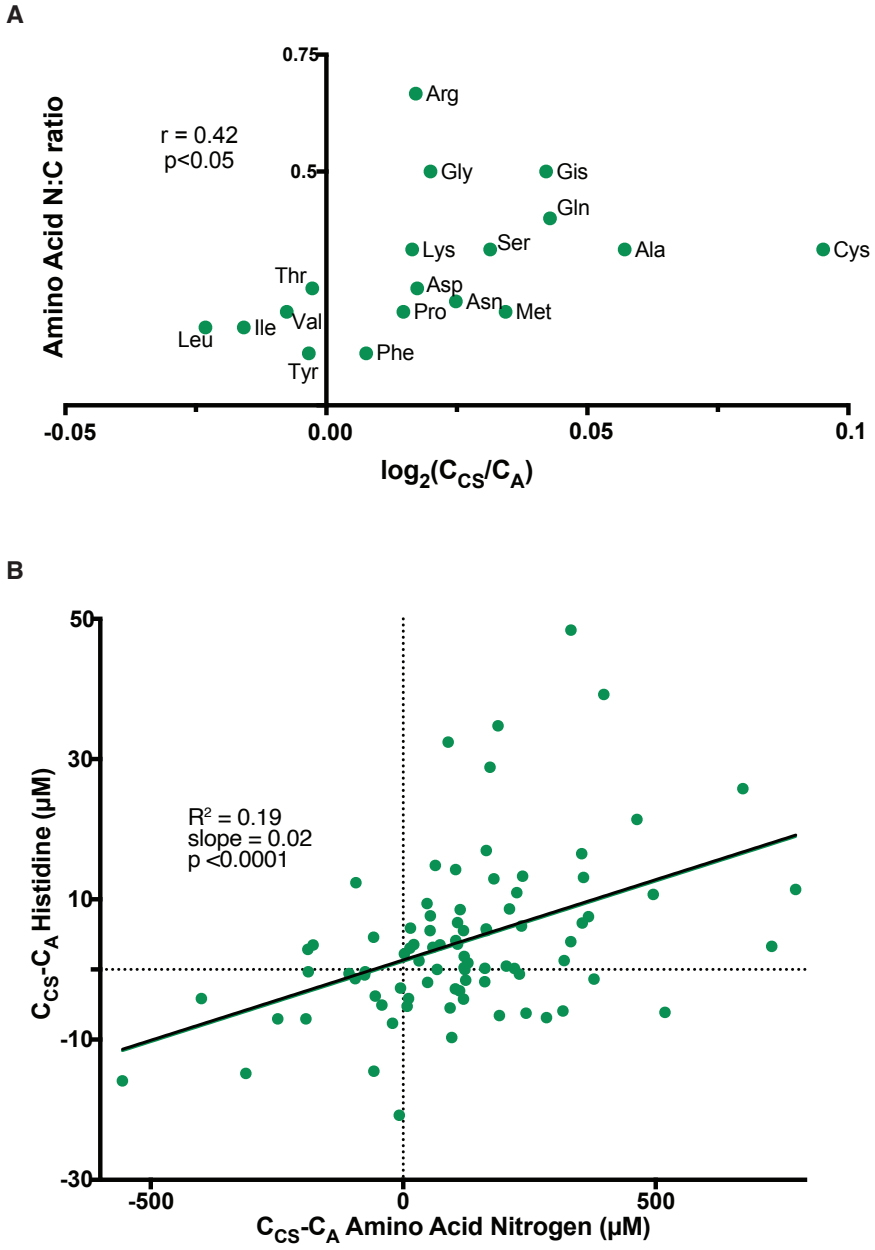

Fig. S5. Preferential release of nitrogen-rich amino acids by the heart
(A) Linear regression of cardiac uptake or release of indicated amino acid ($\log_2 C_{CS}/C_A$) vs. each amino acid's N:C ratio. Data reflect directly measured amino acid concentrations in all patients. P and r values computed by Pearson correlation. **(B)** Linear regression of each patient's cardiac amino acid-derived nitrogen balance vs. cardiac histidine release based on directly quantified amino acids in plasma. Positive values indicate release. p value calculated by F-test.

Fig. S6.

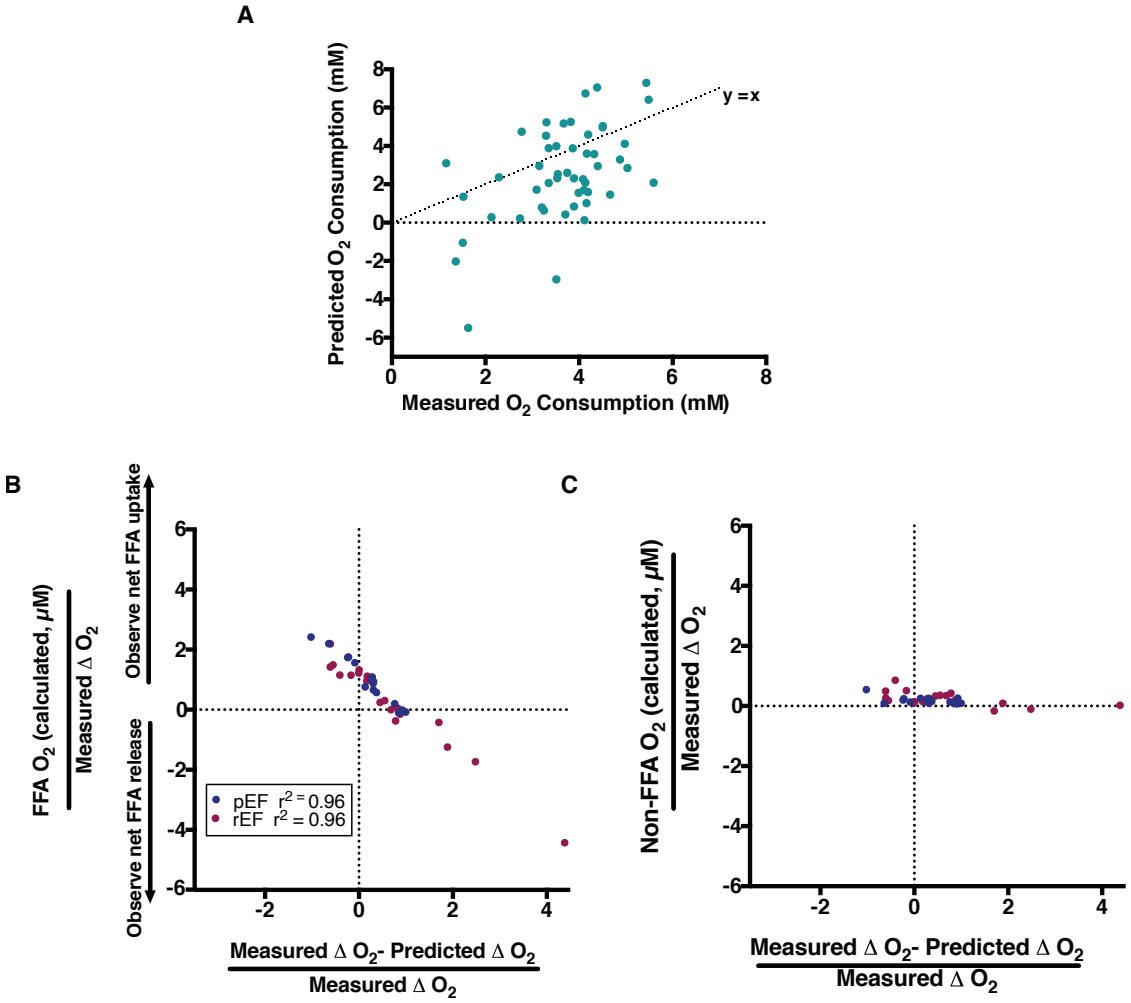

Fig. S6.
A. Comparison of predicted myocardial oxygen consumption based on metabolite use against measured oxygen consumption. Data are from those patients where oxygen consumption values were available (17 pEF, 19 rEF).
B-C. Observed myocardial FFA consumption **(B)** or non-FFA consumption (**C**)**.** In both **B-C,** y axis expressed as fraction of measured ΔO_2 required to combust all consumed FFAs **(B)** or non-FFA species **(C)**; x axis represents proportion of total measured ΔO_2 that is unaccounted for by predicted ΔO_2. Each circle represents a patient.

Fig. S7.

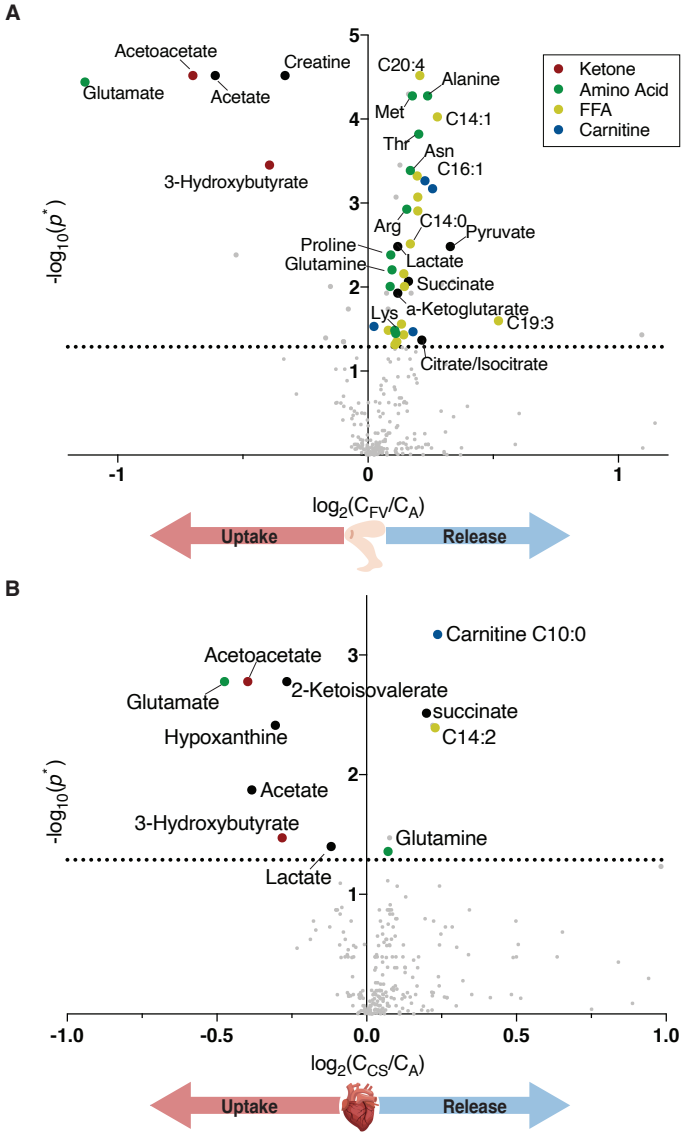

Fig. S7. Metabolite uptake and release profiles in the heart and leg among rEF cohort. Volcano plot depicts metabolite abundance in the femoral vein **(A)** or **(B)** coronary sinus relative to the artery ($\log_2 C_{FV \text{ or } CS}/C_A$) vs. corrected p-value. Metabolites above the dotted line have a corrected p-value < 0.05.

Fig. S8A.

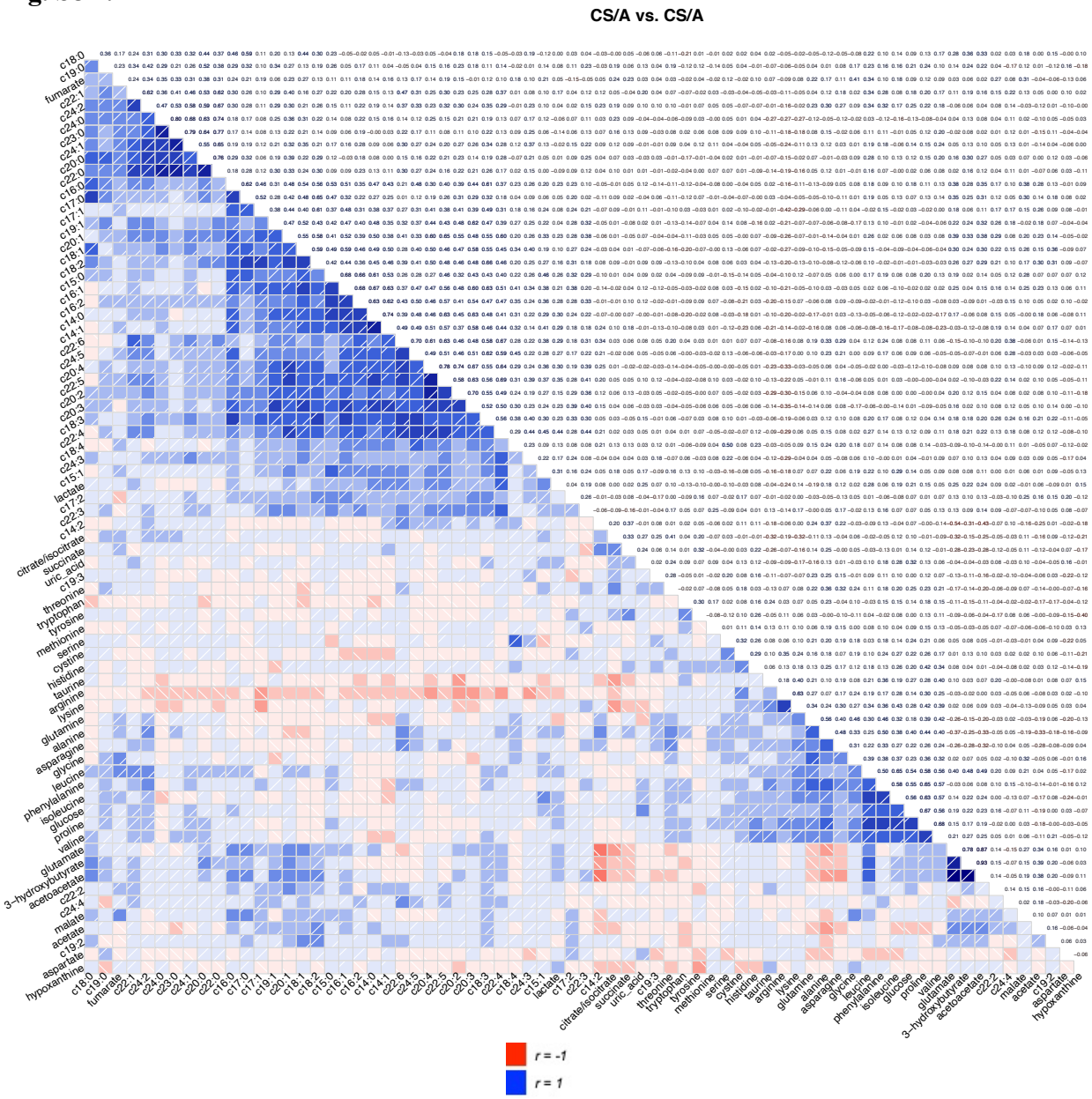

Figure S8A: Comparison of transmyocardial gradients (C_{CS}/C_A) of indicated metabolites across all patients by pearson correlation. Metabolites are ordered by hierarchical clustering. See supplemental data (**Data S2**) for correlation values.

Fig. S8B.

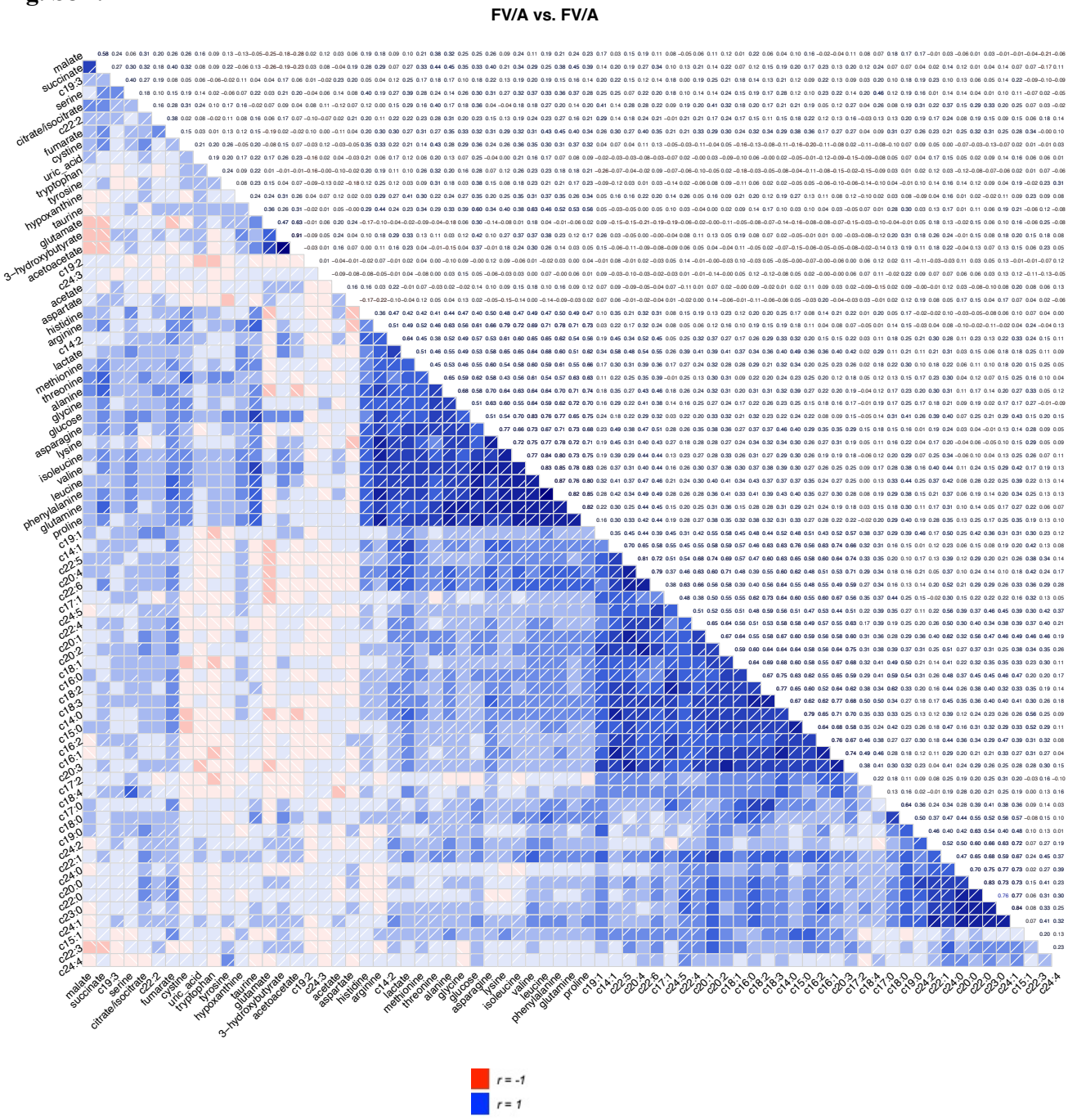

Fig S8B.

Comparison of arteriovenous gradients across the leg (C_{FV}/C_A) of indicated metabolites by pearson correlation. Metabolites are ordered by hierarchical clustering. See supplemental data (**Data S2**) for correlation values.

Fig. S9.

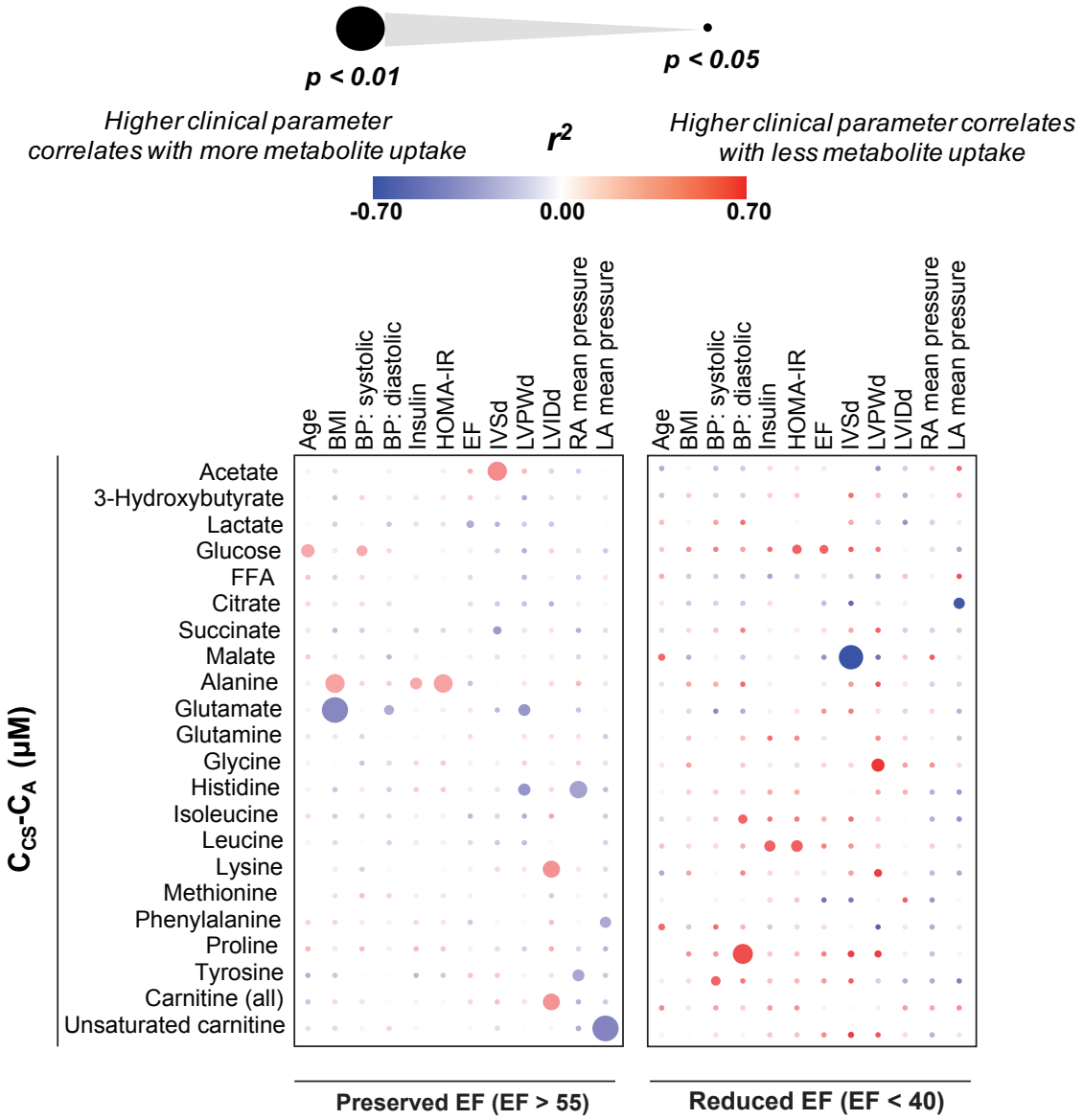

Fig. S9. Relationship between metabolite uptake or release with indicated clinical parameters in pEF and rEF. Shade indicates value of pearson correlation coefficient and size of circle indicates p value (Pearson correlation). Negative (blue) coefficients indicate that a higher clinical parameter value correlates with more metabolite uptake. No p values fell below a Bonferroni-adjusted p value of 0.0002.
Abbreviations: BMI, body mass index; BP, blood pressure; HOMA IR, homeostatic model of insulin resistance; LVEF, left ventricular ejection fraction; IVSd, interventricular septal diameter, diastole; LVIDd, left ventricular internal dimension at end-diastole LVPWd, left ventricular posterior wall thickness at end-diastole; LA pressure, left atrial pressure (mean); RA pressure, right atrial pressure (mean).

Fig. S10.

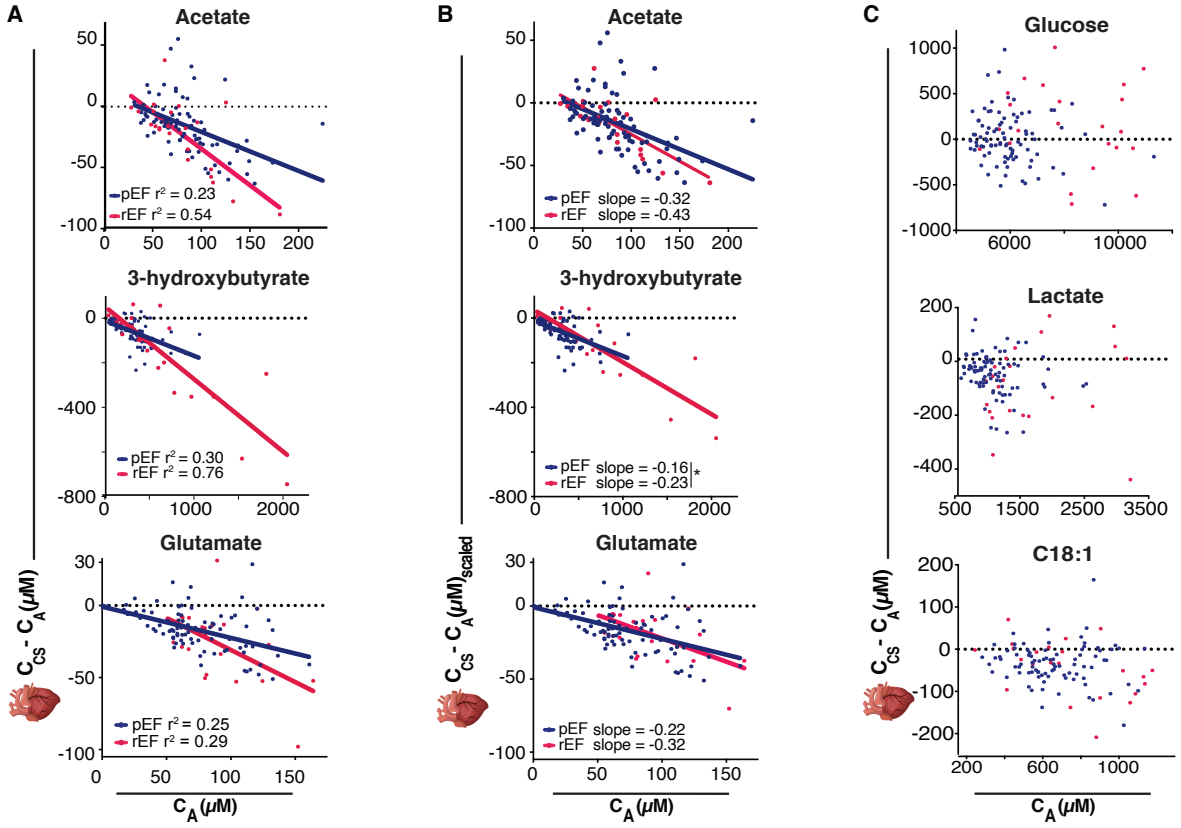

Fig. S10. Total cardiac uptake of acetate, ketones, and glutamate primarily depends upon circulating concentrations in pEF and rEF. (A-B) Arterial concentration (C_A) vs. uptake of select metabolites by the heart (C_{CS}–C_A) before **(A)** and after **(B)** adjustment of metabolite uptake to acetate extraction ($[C_{CS}–C_A]_{scaled}$, see Methods for details). *p<0.05 by ANCOVA **(C)** Arterial concentration (C_A) vs. uptake of select metabolites by the heart (C_{CS}–C_A).

Table S1.

Study Population		
	preserved EF n = 87	reduced EF n = 23
Age – yr, (SD)	63.95 (12.32)	67.35 (9.78)
Female sex – no. (%)	34 (39)	3 (13)
BMI – kg/m²	29.96 (5.85)	29.59 (5.98)
Systolic blood pressure -- mmHg	134.30 (21.42)	124.22 (19.73)
Diastolic blood pressure -- mmHg	81.67 (12.75)	78.78 (11.71)
Resting heart rate -- bpm	79.28 (24.07)	81.96 (23.03)
Hypertension treatment – no. (%)	39 (44.8)	14 (60.9)
Diabetes mellitus treatment -- no. (%)	11 (12.6)	6 (26.1)
Treatment for hyperlipidemia – no. (%)	36 (40.9)	15 (65.2)
Prior PCI – no. (%)	4 (4.59)	4 (17.4%)
Prior CABG– no. (%)	3 (3.45)	6 (26.1%)
Ischemic Cardiomyopathy – no. (%)	0	15 (65.2)
Non-Ischemic Cardiomyopathy -- no. (%)	0	8 (34.8)
Procedure Type		
Ablation of atrial fibrillation	87	20
Ablation of ventricular tachycardia	0	3
Laboratory Values		
Fasting glucose – mg/dL	106.59 (19.49)	106.09 (16.30)
HOMA-IR – (microU * nmol)/dL	2.61 (3.45)	2.45 (1.95)
Atrial Chamber Pressure		
Right atrial mean pressure -- mmHg	10.24 (4.25)	10.63 (4.15)
Left atrial mean pressure -- mmHg	14.68 (7.78)	17.25 (5.26)
Echocardiographic Parameters		
LVEF -- %	58.29 (5.08)	28.69 (9.39)
IVSd -- cm	1.06 (0.20)	1.00 (0.23)
LVPWd -- cm	1.02 (0.19)	1.01 (0.23)
LVIDd -- cm	4.72 (0.63)	6.18 (0.91)

Table S1. Demographic characteristics and clinical parameters of study population.
Abbreviations: LVEF, left ventricular ejection fraction; IVSd, interventricular septal diameter, diastole; LVPWd, left ventricular posterior wall thickness at end-diastole; LVIDd, left ventricular internal dimension at end-diastole.
Data are presented as average (S.D.) unless otherwise noted.

Table S2. Comparison of present study against past arteriovenous metabolomic profiling using the human arm.

	Ivanisevic et al. 2015		Current study	
Metabolite	C_V/C_A	p^*	C_{FV}/C_A	p^*
Alanine	1.17 ± 0.04	0.0003	1.216 ± 0.014	1.02 e-13
Serine	0.93 ± 0.03	0.0144	0.968 ± 0.015	1.44 e-4
Malate	1.76 ± 0.23	0.0012	1.108 ± 0.033	7.17 e-4
Phenylalanine	1.09 ± 0.02	0.0033	1.075 ± 0.009	1.369 e-11
Glutamine	1.17 ± 0.06	0.0259	1.090 ± 0.009	1.41 e-12
Lactate	1.31 ± 0.06	0.0001	1.128 ± 0.011	1.41 e-12
Glutamate	0.33 ± 0.32	0.0001	0.321 ± 0.011	4.30 e-14
Succinate	1.56 ± 0.09	0.0001	1.191 ± 0.014	8.93 e-13
Leucine	0.97 ± 0.03	ns	1.035 ± 0.009	3.52 e-4
Isoleucine	0.97 ± 0.03	ns	1.028 ± 0.010	1.43 e-3
Methionine	1.05 ± 0.06	ns	1.141 ± 0.016	1.55 e-12
Proline	1.02 ± 0.03	ns	1.069 ± 0.009	9.48 e-11
Glycine	1.04 ± 0.02	ns	1.107 ± 0.012	1.68 e-10
Threonine	1.02 ± 0.03	ns	1.098 ± 0.015	6.31 e-8
Asparagine	1.03 ± 0.03	ns	1.111 ± 0.014	2.73 e-10
Histidine	1.02 ± 0.03	ns	1.059 ± 0.014	9.92 e-5
Arginine	1.06 ± 0.05	ns	1.094 ± 0.012	1.80 e-11
Lysine	1.04 ± 0.04	ns	1.068 ± 0.010	5.40 e-12
C22:6	1.10 ± 0.09	ns	1.141 ± 0.013	8.29 e-13
C20:4	1.06 ± 0.07	ns	1.122 ± 0.012	5.01 e-12
Hypoxanthine	1.77 ± 0.39	ns	0.848 ± 0.021	2.16 e-8
Hippuric acid	1.03 ± 0.04	ns	0.803 ± 0.018	1.17 e-11

Table S3. Uptake and release of highly abundant circulating metabolites by the heart and leg in pEF patient population. Red indicates metabolite uptake; blue indicates release. *p* value calculated by 1-sample Wilcoxon test. *p** indicates Benjamini-Hochberg adjusted value.

	Leg (pEF)			Heart (pEF)		
Metabolite	**C_{FV} / C_A**	***p***	***p****	**C_{CS} / C_A**	***p***	***p****
Glucose	0.960 ± 0.008	2.53 e-9	1.69 e-8	1.003 ± 0.005	0.955	0.985
Lactate	1.128 ± 0.01	7.88 e-14	1.41 e-12	0.946 ± 0.008	8.52 e-10	1.70 e-8
3-hydroxybutyrate	0.598 ± 0.017	5.75 e-16	4.30 e-14	0.789 ± 0.015	6.07 e-15	7.90 e-13
Acetoacetate	0.499 ± 0.013	5.56 e-16	4.30 e-14	0.780 ± 0.016	2.45 e-14	1.59 e-12
Acetate	0.730 ± 0.026	1.85 e-11	1.60 e-10	0.833 ± 0.027	5.78 e-8	7.88 e-7
C16:0	1.014 ± 0.010	0.49	0.598	0.962 ± 0.009	7.23 e-6	6.47 e-5
C18:0	0.951 ± 0.011	2.84 e-8	1.57 e-7	0.983 ± 0.010	0.029	0.088
C18:1	1.026 ± 0.010	0.04	0.089	0.936 ± 0.008	1.58 e-10	3.43 e-9
C18:2	1.052 ± 0.011	6.24 e-6	2.42 e-5	0.939 ± 0.008	5.83 e-9	1.08 e-7
C18:3	1.070 ± 0.012	4.71 e-8	2.55 e-7	0.953 ± 0.007	7.35 e-8	9.10 e-7
C20:3	1.064 ± 0.013	5.53 e-6	2.21 e-5	0.965 ± 0.009	8.96 e-5	6.29 e-4
C20:4	1.122 ± 0.012	3.85 e-13	5.01 e-12	0.954 ± 0.009	9.84 e-7	9.84 e-6
C22:6	1.141 ± 0.013	3.51 e-14	8.29 e-13	0.972 ± 0.009	0.002	0.012
Leucine	1.035 ± 0.009	0.0001	3.52 e-4	0.984 ± 0.005	7.85 e-5	5.48 e-4
Isoleucine	1.028 ± 0.010	0.0004	1.43 e-3	0.990 ± 0.005	0.01	0.041
Valine	1.009 ± 0.008	0.27	0.366	0.995 ± 0.005	0.45	0.669
Phenylalanine	1.075 ± 0.009	1.21 e-12	1.37 e-11	1.007 ± 0.004	0.081	0.207
Histidine	1.059 ± 0.014	2.90 e-5	9.922 e-5	1.030± 0.011	0.018	0.062
Lysine	1.068 ± 0.010	6.46 e-11	5.40 e-12	1.012 ± 0.008	0.24	0.449
Threonine	1.098 ± 0.015	1.09 e-8	6.31 e-8	0.999 ± 0.009	0.98	0.99
Methionine	1.141 ± 0.016	9.56 e-14	1.55 e-12	1.023 ± 0.012	0.046	0.128
Alanine	1.216 ± 0.014	2.36 e-15	1.02 e-13	1.042 ± 0.006	2.58 e-8	3.95 e-7
Glycine	1.107 ± 0.012	2.00 e-11	1.68 e-10	1.016 ± 0.010	0.17	0.376
Proline	1.069 ± 0.009	1.02 e-11	9.48 e-11	1.011 ± 0.005	0.007	0.032
Tyrosine	1.024 ± 0.028	0.36	0.469	1.002 ± 0.021	0.744	0.886
Aspartate	0.990 ± 0.020	0.18	0.270	1.010 ± 0.016	0.563	0.744
Glutamate	0.321 ± 0.011	5.56 e-16	4.30 e-14	0.770 ± 0.019	1.87 e-13	9.71 e-12
Arginine	1.094 ± 0.012	2.90 e-10	1.80 e-11	1.019 ± 0.011	0.20	0.101
Serine	0.968 ± 0.015	8.57 e-6	1.55 e-4	1.02 ± 0.015	0.27	0.323
Asparagine	1.111 ± 0.014	3.36 e-11	2.73 e-10	1.019 ± 0.008	0.03	0.089
Glutamine	1.090 ± 0.009	8.14 e-14	1.41 e-12	1.032 ± 0.006	6.06 e-8	7.88 e-7

Table S4. Uptake and release of additional free fatty acids and acylcarnitines by the heart and leg in the pEF population. Red indicates metabolite uptake; blue indicates release. *p* value calculated by 1-sample Wilcoxon test. *p** indicates Benjamini-Hochberg adjusted value.

	Leg (pEF)			Heart (pEF)		
Metabolite	**C_{FV} / C_A**	***p***	***p****	**C_{CS} / C_A**	***p***	***p****
C14:0	**1.157 ± 0.014**	**4.06 e-15**	**1.27 e-15**	**0.950 ± 0.010**	**6.73 e-07**	**6.97 e-06**
C14:1	**1.316 ± 0.017**	**6.61 e-16**	**4.29 e-16**	**0.963 ± 0.010**	**2.94 e-4**	**0.002**
C14:2	**1.119 ± 0.014**	**9.06 e-11**	**6.90 e-10**	**1.13 ± 0.013**	**1.45 e-11**	**3.76 e-10**
C15:0	**1.103 ± 0.015**	**1.87 e-10**	**1.34 e-09**	0.996 ± 0.011	0.206	0.413
C15:1	**1.162 ± 0.019**	**1.20 e-10**	**8.87 e-10**	**0.931 ± 0.014**	**2.62 e-07**	**2.83 e-06**
C16:1	**1.173 ± 0.0149**	**3.66 e-15**	**1.31 e-13**	**0.943 ± 0.009**	**8.50 e-09**	**1.38 e-07**
C16:2	**1.153 ± 0.016**	**1.14 e-12**	**3.02 e-13**	**0.958 ± 0.012**	**4.9 e-4**	**2.9 e-3**
C17:0	1.019 ± 0.012	0.89	0.92	1.003 ± 0.011	0.76	0.89
C17:1	**1.083 ± 0.013**	**5.23 e-07**	**2.51 e-06**	**0.951 ± 0.0114**	**2.7 e-4**	**0.0017**
C17:2	**1.076 ± 0.016**	**8.96 e-05**	**2.87 e-4**	**0.942 ± 0.0144**	**2.47 e-05**	**1.99 e-4**
C18:3	**1.078 ± 0.011**	**4.71 e-08**	**2.54 e-07**	**0.956 ± 0.007**	**7.35 e-08**	**9.06 e-07**
C18:4	**1.140 ± 0.023**	**1.48 e-07**	**7.69 e-07**	1.022 ± 0.017	0.52	0.71
C20:0	**0.940 ± 0.013**	**1.34 e-09**	**9.11 e-09**	**0.959 ± 0.013**	**1.21 e-07**	**1.42 e-06**
C20:1	0.989 ± 0.011	**0.0091**	**0.02**	**0.914 ± 0.009**	**7.83 e-12**	**2.39 e-10**
C20:2	**1.033 ± 0.011**	**0.024**	**0.049**	**0.959 ± 0.008**	**1.87 e-06**	**1.80 e-05**
C22:0	**0.963 ± 0.02**	**2.71 e-05**	**9.36 e-05**	**0.962 ± 0.017**	**7.46 e-06**	**6.44 e-05**
C22:1	**0.952 ± 0.013**	**1.40 e-05**	**5.16 e-05**	**0.937 ± 0.013**	**6.45 e-09**	**1.11 e-07**
C22:2	**0.984 ± 0.03**	**0.0030**	**0.0076**	**0.973 ± 0.024**	**0.0049**	**0.022**
C22:4	**1.077 ± 0.012**	**5.47 e-07**	**2.58 e-06**	**0.939 ± 0.010**	**3.89 e-08**	**5.60 e-07**
C22:5	**1.121 ± 0.013**	**4.72 e-12**	**4.71 e-11**	**0.966 ± 0.011**	**5.40 e-4**	**0.0030**
carnitine C2	**0.949 ± 0.009**	**3.68 e-09**	**2.27 e-08**	0.994 ± 0.006	0.35	0.57
carnitine C3	0.991 ± 0.015	0.043	0.085	1.012 ± 0.016	0.53	0.71
carnitine C4	0.994 ± 0.012	0.029	0.059	1.014 ± 0.012	0.38	0.59
carnitine C5:0	**1.051 ± 0.016**	**3.58 e-05**	**1.20 e-4**	0.988 ± 0.010	0.51	0.71
carnitine C6:0	**0.847 ± 0.013**	**1.32 e-13**	**1.89 e-12**	0.983 ± 0.012	0.017	0.060
carnitine C8:0	**1.127 ± 0.016**	**9.07 e-12**	**8.70 e-11**	**1.032 ± 0.012**	**0.0032**	**0.015**
carnitine C8:1	**0.843 ± 0.015**	**1.78 e-11**	**1.59 e-10**	1.011 ± 0.012	0.89	0.95
carnitine C10:0	**1.134 ± 0.014**	**1.49 e-13**	**2.04 e-12**	**1.028 ± 0.011**	**0.0054**	**0.024**
carnitine C10:1	**1.079 ± 0.015**	**2.07 e-06**	**8.78 e-06**	**1.034 ± 0.011**	**0.012**	**0.048**
carnitine C10:2	**1.047 ± 0.016**	**0.0039**	**0.0097**	1.031 ± 0.014	0.020	0.066
carnitine C10:3	**1.040 ± 0.021**	**0.021**	**0.045**	**1.074 ± 0.036**	**0.0024**	**0.012**
carnitine C14:0	**0.956 ± 0.023**	**0.0098**	**0.022**	1.006 ± 0.023	0.89	0.95
carnitine C14:1	**0.964 ± 0.017**	**9.85 e-4**	**0.003**	0.983 ± 0.018	0.11	0.26
carnitine C14:2	**0.945 ± 0.013**	**1.94 e-6**	**8.38 e-6**	0.985 ± 0.011	0.26	0.46
carnitine C16:0	**0.966 ± 0.023**	**0.009**	**0.021**	1.028 ± 0.028	0.65	0.82
carnitine C16:2	0.961 ± 0.031	0.063	0.12	1.001 ± 0.027	0.57	0.75
carnitine C18:0	**0.988 ± 0.019**	**0.021**	**0.044**	1.017 ± 0.021	0.86	0.94
carnitine C18:1	**0.973 ± 0.013**	**0.003**	**0.006**	0.985 ± 0.008	0.16	0.36
carnitine C18:2	0.996 ± 0.018	0.39	0.49	1.017 ± 0.014	0.54	0.73

Table S5. Measured concentrations in arterial plasma of indicated metabolites. Data are average concentration (μM) ± standard error in the artery (C_A). P-values are the result of a Mann-Whitney rank sum test. Those p-values listed in red fall below a Bonferroni-adjusted critical value of 1.667 e-3.

	C_A (μM)		
	Preserved EF	**Reduced EF**	***p***
Glucose	6057.47 ± 133.89	8208.77 ± 394.53	**1.0374 e-5**
Lactate	1132.06 ± 34.86	1555.57 ± 145.96	**1.179 e-3**
3-hydroxybutyrate	305.22 ± 20.71	655.03 ± 114.26	**5.141 e-3**
Acetate	83.62 ± 3.65	79.68 ± 7.51	**0.593**
C16:0	365.57 ± 10.77	402.83 ± 27.15	**0.0980**
C18:0	93.36 ± 3.46	93.67 ± 3.05	**0.989**
C18:1	572.08 ± 17.12	634.85 ± 47.71	**0.225**
C18:2	590.58 ± 15.07	523.32 ± 44.48	**0.165**
C20:4	10.22 ± 0.38	11.68 ± 0.99	**0.177**
C22:6	8.81 ± 0.51	8.08 ± 1.45	**0.637**
Glutamine	553.27 ± 8.34	791.74 ± 45.40	**2.902 e-5**
Leucine	155.25 ± 4.07	178.92 ± 7.13	**3.846 e-3**
Isoleucine	78.45 ± 2.06	90.42 ± 3.60	**3.846 e-3**
Glutamate	70.41 ± 3.81	88.66 ± 6.42	**1.916 e-2**
Histidine	115.91 ± 2.64	127.85 ± 4.64	**2.973 e-2**
Phenylalanine	73.80 ± 1.97	81.46 ± 3.35	**4.931 e-2**
Threonine	116.75 ± 3.20	104.03 ± 5.97	**6.204 e-2**
Valine	250.34 ± 5.81	270.44 ± 10.81	**8.639 e-2**
Alanine	252.47 ± 8.78	282.41 ± 14.50	**8.909 e-2**
Asparagine	47.26 ± 1.17	52.98 ± 3.32	**0.115**
Arginine	130.03 ± 6.72	114.05 ± 10.19	**0.168**
Proline	141.79 ± 3.93	131.23 ± 9.17	**0.289**
Tryptophan	49.65 ± 1.42	46.41 ± 3.30	**0.298**
Tyrosine	73.03 ± 2.57	78.04 ± 4.83	**0.364**
Serine	63.36 ± 1.59	66.07 ± 3.39	**0.491**
Lysine	215.31 ± 5.69	225.02 ± 13.25	**0.542**
Taurine	43.85 ± 1.17	43.61 ± 1.45	**0.980**
Hypoxanthine	12.47 ± 1.01	16.97 ± 6.40	**0.434**
Uric Acid	301.94 ± 12.34	361.67 ± 29.41	**0.145**

Table S6. Uptake and release of indicated metabolites by the heart. Data are average measured uptake or release (μM) ± standard error of indicated metabolites by the heart in patients with preserved or reduced ejection fraction (EF). Average flux in nmol/min/g was calculated assuming a coronary blood flow of 0.8 ml/min/g. P-values are the result of a 1-sample Wilcoxon rank-sum test where $\mu_0 |C_{CS}-C_A| = 0$.

	$C_{CS} - C_A$					
	Preserved EF			Reduced EF		
	μM	nmol/min/g	*p*	μM	nmol/min/g	*p*
Glucose	10.49 ± 29.89	8.39	0.9829	145.24 ± 94.42	116.19	0.14
Lactate	**-54.05 ± 7.97**	-43.24	**<0.0001**	**-94.94 ± 30.54**	-75.95	**0.006**
3-hydroxybutyrate	**-61.31 ± 5.84**	-49.05	**<0.0001**	**-163.61 ± 43.61**	-130.89	**0.0002**
Acetate	**-15.34 ± 3.25**	-12.27	**<0.0001**	**-22.83 ± 6.16**	-18.26	**0.0002**
C16:0	**-14.33 ± 3.53**	-11.46	**<0.0001**	-12.05 ± 11.42	-9.64	0.08
C18:0	**-1.65 ± 1.05**	-1.32	**0.01**	3.82 ± 2.43	3.06	0.48
C18:1	**-33.81 ± 5.02**	-27.05	**<0.0001**	**-44.20 ± 13.76**	-35.36	**0.004**
C18:2	**-33.52 ± 4.95**	-26.82	**<0.0001**	**-22.09 ± 10.08**	-17.67	**0.02**
C20:4	**-0.46 ± 0.09**	-0.37	**<0.0001**	-0.10 ± 0.23	-0.08	0.39
C22:6	**-0.20 ± 0.08**	-0.16	**0.006**	0.44 ± 0.23	0.35	0.22
Hypoxanthine	**-3.30 ± 0.27**	-2.64	**<0.0001**	**-2.92 ± 1.20**	-2.34	**0.01**
Uric Acid	10.19 ± 0.42	8.15	0.90	130.80 ± 75.97	104.64	0.94
Glutamine	**17.61 ± 3.18**	14.09	**<0.0001**	**34.04 ± 12.57**	27.23	**0.004**
Glutamate	**-16.08 ± 1.64**	-12.86	**<0.0001**	**-25.84 ± 5.32**	-20.67	**<0.0001**
Phenylalanine	0.72 ± 0.35	0.58	0.17	1.83 ± 1.00	1.46	0.13
Threonine	0.22 ± 1.02	0.18	0.92	4.58 ± 2.31	3.66	0.07
Histidine	**4.34 ± 1.29**	3.47	**0.02**	**5.53 ± 4.39**	4.42	**0.04**
Lysine	3.32 ± 1.93	2.66	0.27	**7.40 ± 5.58**	5.92	**0.01**
Arginine	2.64 ± 1.62	2.11	0.14	**3.23 ± 3.39**	2.58	**0.01**
Isoleucine	**-0.68 ± 0.34**	-0.54	**0.009**	0.27 ± 1.31	0.22	0.89
Leucine	**-2.36 ± 0.76**	-1.89	**<0.0001**	-0.01 ± 2.10	-0.01	0.87
Tryptophan	**2.87 ± 3.27**	2.30	0.01	**-0.41 ± 4.38**	-0.33	**0.03**
Valine	-0.44 ± 1.13	-0.35	0.43	7.60 ± 3.86	6.08	0.11
Proline	**2.04 ± 0.70**	1.63	**0.005**	**2.81 ± 1.89**	2.25	**0.04**
Tyrosine	-0.22 ± 1.53	-0.18	0.66	-5.10 ± 3.63	-4.08	0.19
Alanine	**11.49 ± 1.86**	9.19	**<0.0001**	**15.33 ± 6.33**	12.26	0.02
Asparagine	**0.93 ± 0.41**	0.74	**0.04**	**2.71 ± 1.12**	2.17	**0.02**
Serine	1.66 ± 1.21	1.33	0.29	0.84 ± 1.44	0.67	0.54
Taurine	**0.86 ± 0.38**	0.69	**0.01**	0.96 ± 0.91	0.77	0.41

Table S7. Uptake and release of indicated metabolites by the leg. Data are average measured uptake or release (μM) ± standard error of indicated metabolites by the leg in patients with preserved or reduced ejection fraction (EF). Average flux in nmol/min/g was calculated assuming average blood flow of 0.035 ml/min/g in the leg. *p* values are the result of a 1-sample Wilcoxon rank-sum test where $\mu_0 |C_{FV}-C_A| = 0$.

	$C_{FV} - C_A$					
	Preserved EF			Reduced EF		
	μM	nmol/min/g	*p*	μM	μmol/min/g	*p*
Glucose	-231.94 ± 50.05	-8.12	<0.0001	-272.66 ± 132.42	-9.54	0.01
Lactate	127.20 ± 13.54	4.45	<0.0001	133.02 ± 33.47	4.66	0.0006
3-hydroxybutyrate	-108.77 ± 6.96	-3.81	<0.0001	-153.74 ± 33.75	-5.38	<0.0001
Acetate	-25.26 ± 2.90	-0.88	<0.0001	-30.41 ± 5.25	-1.06	<0.0001
C16:0	5.91 ± 3.84	0.21	0.80	15.66 ± 13.95	0.55	0.68
C18:0	-4.22 ± 0.99	-0.15	<0.0001	-0.50 ± 2.65	-0.018	0.85
C18:1	15.69 ± 5.43	0.55	0.11	18.59 ± 17.44	0.65	0.39
C18:2	30.76 ± 6.38	1.08	<0.0001	12.84 ± 12.68	0.45	0.17
C20:4	1.20 ± 0.13	0.042	<0.0001	1.75 ± 0.34	0.061	<0.0001
C22:6	1.19 ± 0.13	0.042	<0.0001	0.76 ± 0.33	0.027	<0.0001
Hypoxanthine	-1.89 ± 0.15	-0.066	0.0002	-2.93 ± 1.77	-0.10	0.09
Uric Acid	4.83 ± 0.20	0.17	0.36	48.11 ± 49.04	1.68	0.16
Glutamine	49.84 ± 4.96	1.74	<0.0001	44.71 ± 13.24	1.56	0.002
Glutamate	-47.82 ± 2.72	-1.67	<0.0001	-49.84 ± 6.50	-1.74	<0.0001
Phenylalanine	5.75 ± 0.67	0.20	<0.0001	4.96 ± 1.60	0.17	0.003
Threonine	11.54 ± 1.63	0.40	<0.0001	15.13 ± 3.73	0.53	0.0001
Histidine	7.35 ± 1.50	0.26	<0.0001	4.37 ± 2.99	0.15	0.13
Lysine	15.40 ± 2.43	0.54	<0.0001	16.58 ± 5.56	0.58	0.004
Arginine	11.73 ± 1.74	0.41	<0.0001	11.49 ± 3.43	0.40	0.0002
Isoleucine	2.51 ± 0.79	0.088	0.0004	2.89 ± 2.32	0.10	0.11
Leucine	5.83 ± 1.39	0.20	0.0002	5.99 ± 3.49	0.21	0.11
Tryptophan	4.76 ± 3.83	0.17	0.16	-3.54 ± 3.55	-0.12	0.07
Valine	3.45 ± 2.04	0.12	0.61	5.79 ± 5.17	0.20	0.75
Proline	10.22 ± 1.19	0.36	<0.0001	8.66 ± 3.13	0.30	0.0003
Tyrosine	1.45 ± 1.72	0.051	0.33	-0.74 ± 4.07	-0.026	0.32
Alanine	57.88 ± 4.45	2.03	<0.0001	50.56 ± 10.20	1.77	<0.0001
Asparagine	5.09 ± 0.57	0.18	<0.0001	6.47 ± 1.34	0.23	<0.0001
Serine	-2.37 ± 1.15	-0.083	<0.0001	-2.18 ± 1.91	-0.076	0.23
Taurine	-0.46 ± 0.46	-0.016	0.02	0.88 ± 1.25	0.031	0.82

Table S8. Substrate-specific contribution to cardiac ATP generation in patients with pEF or rEF. Corresponds to Fig. 3B. See Methods for details of calculations.

	Contribution to cardiac ATP generation (%)	
	Preserved EF	Reduced EF
C18:1	14.95	18.67
C18:2	19.19	9.21
C16:0	5.52	4.55
C18:3	1.53	0.48
C20:3	1.25	0.22
C18:0	0.81	-
C20:1	0.61	0.29
C22:5	0.55	-
C22:4	0.50	0.14
C16:1	0.35	0.33
C14:0	0.31	0.41
C20:2	0.27	0.10
C20:4	0.21	0.05
C17:1	0.18	0.15
C22:1	0.15	0.02
C22:0	0.11	-
C20:0	0.10	-
C22:6	0.10	-
C15:0	0.05	0.14
C22:2	0.03	0.08
C14:1	0.03	0.03
C22:3	0.02	0.01
C19:0	0.02	-
C24:1	0.01	-
C19:1	0.01	0.01
C15:1	0.002	0.002
C17:0	0.001	0.003
3-hydroxybutyrate	4.86	13.12
Acetoacetate	1.53	3.28
Lactate	2.79	5.00
Acetate	0.17	0.24
2-Ketoisocaproate	0.09	0.19
2-Ketoisovalerate	0.12	0.16
Carnitine C18:1	0.002	-
Glutamate	1.10	1.61
Leucine	0.90	1.09
Isoleucine	0.62	0.96
Valine	0.45	0.01
Lysine	0.38	0.53
Aspartate	0.32	0.62
Threonine	0.30	0.20
Asparagine	0.24	0.36
Proline	0.14	0.42
Arginine	0.09	0.35
Serine	0.07	
Methionine	0.01	0.08
LpFA/uncounted FFA	44.0	36.5

Table S9. Uptake and release of indicated metabolites by the heart. Blue indicates significant release. P-values for C_{CS}/C_A are the result of a 1-sample Wilcoxon rank-sum test with Benjamini-Hochberg correction (*). P-values for comparison of pEF vs. rEF were derived from a student's t-test; no p values for this test were below a Bonferroni-adjusted critical value of 0.0002.

	Preserved EF			Reduced EF			Comparison
	C_{CS} / C_A	SEM	*p**	C_{CS} / C_A	SEM	*p**	*p*
carnitine	1.003	5.97 e-3	0.964	1.005	0.015	0.992	0.865
acetyl-carnitine	0.993	5.63 e-3	0.567	1.021	0.019	0.912	0.175
propionyl-carnitine	1.012	0.015	0.716	1.055	0.032	0.264	0.240
butyryl-carnitine	1.014	0.011	0.599	1.047	0.021	0.264	0.170
carnitine c5:0	0.990	9.49 e-3	0.713	1.008	0.021	0.851	0.450
carnitine c5:1	1.015	0.027	0.860	1.047	0.039	0.707	0.502
carnitine c6:0	0.983	0.011	0.060	1.069	0.025	0.160	0.003
carnitine c8:0	**1.032**	**0.012**	**0.015**	1.037	0.019	0.211	0.824
carnitine c8:1	1.010	0.011	0.954	1.181	0.131	0.483	0.207
carnitine c10:0	**1.030**	**0.010**	**0.024**	**1.100**	**0.022**	**8.48 e-4**	0.008
carnitine c10:1	**1.033**	**0.010**	**0.048**	1.062	0.022	0.146	0.256
carnitine c10:2	1.031	0.014	0.066	1.025	0.024	0.781	0.843
carnitine c10:3	**1.074**	**0.036**	**0.012**	0.995	0.014	0.912	0.045
carnitine c12:0	1.037	0.029	0.546	1.054	0.028	0.261	0.771
carnitine c12:1	1.012	0.011	0.421	1.008	0.016	0.996	0.843
carnitine c12:2	1.045	0.025	0.394	1.071	0.031	0.195	0.519
carnitine c14:0	1.006	0.020	0.955	1.007	0.035	0.963	0.981
carnitine c14:1	0.983	0.018	0.264	1.019	0.030	0.928	0.311
carnitine c14:2	0.985	0.011	0.463	1.045	0.028	0.653	0.052
carnitine c16:0	1.028	0.028	0.822	1.027	0.065	0.928	0.985
carnitine c16:1	1.017	0.037	0.750	1.033	0.028	0.928	0.737
carnitine c16:2	1.001	0.027	0.292	1.120	0.088	0.684	0.208
carnitine c18:0	1.017	0.021	0.940	1.078	0.072	0.992	0.421
carnitine c18:1	0.985	8.41 e-3	0.364	1.073	0.035	0.085	0.024
carnitine c18:2	1.017	0.014	0.728	1.033	0.028	0.747	0.617
carnitine c20:0	1.046	0.039	0.989	1.167	0.128	0.659	0.377
carnitine c24:0	1.010	0.015	0.701	0.959	0.050	0.685	0.335
citrate	1.050	0.022	0.077	1.072	0.041	0.406	0.644
α-ketoglutarate	0.979	0.019	0.369	1.037	0.020	0.365	0.038
succinate	**1.101**	**0.012**	**2.72e-11**	**1.148**	**0.049**	**3.06 e-3**	0.361
fumarate	1.042	0.017	0.088	1.040	0.037	0.558	0.957
malate	1.097	0.059	0.225	0.959	0.038	0.707	0.052

Table S10. Uptake and release of indicated metabolites by the leg. Data are average estimated uptake or release (μM) ± standard error of indicated metabolites by the leg in patients with preserved or reduced ejection fraction (EF). *p**: 1-sample Wilcoxon rank-sum test with Benjamini-Hochberg correction. Red indicates significant uptake, blue indicates significant release. P-values for comparison of pEF vs. rEF were derived from a student's t-test; no p values for pEF vs. rEF comparison fell below a Bonferroni-adjusted critical value of 0.0002.

	C_{FV} - C_A						
	Preserved EF			Reduced EF			Comparison
	C_{FV} / C_A	SEM	*p**	C_{FV} / C_A	SEM	*p**	*p*
carnitine	1.013	7.88 e-3	0.218	1.021	0.015	0.229	0.628
acetyl-carnitine	0.948	8.80 e-3	2.25 e-8	1.001	0.022	0.234	0.035
propionyl-carnitine	0.987	0.014	0.084	1.017	0.026	0.992	0.333
butyryl-carnitine	0.991	0.011	0.059	1.015	0.023	0.843	0.362
carnitine c5:0	1.052	0.015	1.20 e-4	1.034	0.019	0.348	0.456
carnitine c5:1	0.980	0.025	0.627	1.044	0.023	0.121	0.06
carnitine c6:0	0.850	0.013	1.88 e-12	0.948	0.026	0.037	0.002
carnitine c8:0	1.127	0.015	8.64 e-11	1.092	0.019	6.82 e-4	0.146
carnitine c8:1	0.844	0.014	1.58 e-10	1.057	0.150	0.042	0.172
carnitine c10:0	1.133	0.014	2.02 e-12	1.115	0.026	8.51 e-4	0.558
carnitine c10:1	1.077	0.014	8.71 e-6	1.063	0.025	0.076	0.629
carnitine c10:2	1.048	0.015	9.64 e-3	1.036	0.033	0.825	0.736
carnitine c10:3	1.039	0.020	0.044	1.020	0.017	0.542	0.469
carnitine c12:0	1.042	0.024	0.358	1.048	0.029	0.646	0.874
carnitine c12:1	1.005	0.013	0.813	1.022	0.019	0.743	0.465
carnitine c12:2	1.063	0.022	0.048	1.088	0.029	0.039	0.49
carnitine c14:0	0.956	0.023	0.022	0.953	0.028	0.276	0.949
carnitine c14:1	0.964	0.017	2.69 e-3	0.999	0.022	0.799	0.22
carnitine c14:2	0.945	0.013	8.32 e-6	1.015	0.025	0.925	0.018
carnitine c16:0	0.966	0.023	0.021	0.969	0.054	0.843	0.967
carnitine c16:1	0.968	0.036	0.114	1.001	0.046	0.445	0.583
carnitine c16:2	0.961	0.031	5.89 e-3	1.086	0.063	0.979	0.084
carnitine c18:0	0.988	0.019	0.044	1.054	0.049	0.992	0.222
carnitine c18:1	0.973	0.013	6.44 e-3	1.059	0.030	0.234	0.015
carnitine c18:2	0.996	0.018	0.493	1.007	0.028	0.911	0.735
carnitine c20:0	1.002	0.032	0.804	1.014	0.062	0.849	0.871
carnitine c24:0	0.982	0.016	0.234	1.058	0.044	0.542	0.117
citrate	1.209	0.033	2.08 e-8	1.161	0.061	0.042	0.497
α-ketoglutarate	1.010	0.032	0.537	1.086	0.029	0.012	0.081
succinate	1.191	0.014	8.83 e-13	1.118	0.034	8.55 e-3	0.056
fumarate	1.117	0.023	6.27 e-5	1.078	0.035	0.250	0.372
malate	1.108	0.033	7.08 e-4	1.001	0.034	0.849	0.028

Data S1. Summary of C_{CS}/C_A and C_{FV}/C_A for all metabolites from all patients.

Column Headings:

FV_over_A_pEF or **CS_over_A_pEF:** Mean C_{FV}/C_A or C_{CS}/C_A for indicated metabolite from preserved EF (pEF) cohort.

FV_over_A_pEF_sem or **CS_over_A_pEF_sem:** Standard error of C_{FV}/C_A or C_{CS}/C_A for indicated metabolite from pEF cohort.

Raw_p_FV_over_A_pEF or **Raw_P_CS_over_A_pEF:** Raw p value from Wilcoxon signed rank test with H_0 : C_{FV}/C_A or $C_{CS}/C_A = 1$, pEF cohort.

BH_p_FV_over_A_pEF or **BH_p_CS_over_A_pEF:** Benjamini-Hochberg corrected p value, pEF cohort.

FV_over_A_rEF or **CS_over_A_rEF:** Mean C_{FV}/C_A or C_{CS}/C_A for indicated metabolite from reduced EF (rEF) cohort.

FV_over_A_sem_rEF or **CS_over_A_sem_rEF:** Standard error of C_{FV}/C_A or C_{CS}/C_A for indicated metabolite from rEF cohort.

Raw_p_FV_over_A_rEF or **Raw_p_CS_over_A_rEF:** Raw p value from Wilcoxon signed rank test with H_0: C_{FV}/C_A or $C_{CS}/C_A = 1$, rEF cohort.

BH_p_FV_over_A_rEF or **BH_p_CS_over_A_rEF:** Benjamini-Hochberg corrected p value, rEF cohort.

"NA" values where FV or CS/A ratio available from <20 patients.

Data S2. Summary of *p* and r^2 values for metabolite extraction correlations (corresponds to **Fig S7A-B)**.

Tab 1 ("CS_A vs. CS_A correlation"): Corresponds to Fig S7A. Each row corresponds to a metabolite pairing.

Tab 2 ("FV_A vs. FV_A correlation"): Corresponds to Fig S7B. Each row corresponds to a metabolite pairing.

Column "cor": r^2 of correlation between compound 1 and compound 2.

Column "p": p value of correlation between compound 1 and compound 2.

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