Tissue metabolic profiling shows that saccharopine accumulates during renal ischemic-reperfusion injury, while kynurenine and itaconate accumulate in renal allograft rejection

Ulf H. Beier,[1,7] Erum A. Hartung,[1,7] Seth Concors,[2] Paul T. Hernandez,[2] Zhonglin Wang,[2], Caroline Perry,[3] Joseph A. Baur,[3] Michelle R. Denburg,[1], Wayne W. Hancock,[4] Terence P. Gade[5,6], Matthew H. Levine[2]*

[1]Division of Nephrology, Department of Pediatrics, Children's Hospital of Philadelphia and Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104; [2]Department of Surgery, Penn Transplant Institute, Perelman School of Medicine, Children's Hospital of Philadelphia and University of Pennsylvania, Philadelphia, PA 19104; [3]Department of Physiology and Institute of Diabetes, Obesity, and Metabolism, University of Pennsylvania, Philadelphia, PA 19104; [4]Division of Transplant Immunology, Department of Pathology and Laboratory Medicine, and Biesecker Center for Pediatric Liver Disease, Children's Hospital of Philadelphia and University of Pennsylvania, Philadelphia, PA 19104. [5]Department of Radiology, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, 19104. [6]Department of Cancer Biology, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, 19104.
[7]Authors contributed equally.

Running headline: *Metabolites in renal IRI vs. allograft rejection*

*Correspondence: Matthew H. Levine, MD, PhD, 3400 Spruce Street, 2 Ravdin Courtyard, Transplant Surgery, Hospital of the University of Pennsylvania, Philadelphia, PA 19104, P: 215-662-7367, F: 215-615-4900, matthew.levine@uphs.upenn.edu

Detailed Methods

Mice

We purchased C57BL/6 and BALB/c mice rom The Jackson Laboratory (Bar Harbor, ME). Mice were housed under specific-pathogen-free conditions were studied using protocols approved by the Institutional Animal Care and Use Committees of the Children's Hospital of Philadelphia and University of Pennsylvania (19–000561 and 17-000954). All animals received the same chow containing 26.101% fat, and 19.752% protein with 1.05% lysine (Mouse Diet 5015, LabDiet, St. Louis, MO).

Renal allografting and organ harvest

Organ procurement and transplant procedure was performed per published techniques (Levine et al, 2016; Wang et al, 2009). In this study, we procured kidneys from 6-8 week old female C57BL/6 (murine major histocompatibility complex (MHC) haplotype $H-2^b$) donor mice, and transplanted them to MHC-mismatched 6-8 week female BALB/c (MHC: $H-2^d$) recipient mice. Recipient native kidneys were not removed at the time of transplant to avoid confounding effects from impaired renal function on metabolism. Our previous studies have shown that peak acute cellular rejection (ACR) occurs around 14 days post-transplant in this model, based on histology and, in recipient mice that have undergone native nephrectomy, creatinine and blood urea nitrogen measurements (Levine et al, 2016). We therefore chose to harvest kidney allografts undergoing ACR ($H-2^b$) and control native kidneys (NK, $H-2^d$) at 14 days post-transplant in this study.

Warm ischemia-reperfusion injury model and organ harvest

The ischemia-reperfusion injury (IRI) model has been described previously (Levine et al, 2015). In this study, we worked with 8-12 week old female C57BL/6 mice ($H-2^b$). Mice were anesthetized with

pentobarbital (65 mg/kg intraperitoneally) and placed in a temperature-controlled container with a surgical microscope and a heating pad. Throughout the surgery, core body temperature was continuously monitored and maintained at 36.0 ± 0.5 °C. After incision, one renal pedicle was exposed and clamped for 28 min with a microvascular clip (Roboz Surgical Instrument Co., Gaithersburg, MD). Successful ischemia was confirmed by color change. After the clamp was released, the animal received 100 mL/kg saline subcutaneously for hydration. The kidneys were harvested 24 hours after the surgery (Levine et al, 2015), with the clamped kidney serving as IRI kidney, and the unclamped kidney as control (CTR).

Ultrahigh performance liquid chromatography-tandem mass spectrometry

After harvesting, the ACR, NK, CTR and IRI kidneys were snap frozen in liquid nitrogen and then stored at –80 °C, and then shipped to Metabolon (Morrisville, NC) for metabolite extraction and ultrahigh performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). The kidney samples were subjected to an intact sample extraction procedure. The samples were weighed and then 80% methanol was added at a constant weight by volume ratio (1:30). Metabolite extraction included protein precipitation and removal with methanol, shaking and centrifugation. All 40 samples were processed in one plate to avoid batch effects, and analyzed together with three process blanks, a Metabolon plasma sample, and four 'client matrix' controls (samples pooled form the forty specimens) to ensure consistent results. The 'client matrix' samples were used to are used to monitor the run over the course of the plate to ensure that the same peaks are monitored as the beginning middle, and end of each run so any drift in elution time can be monitored. This was in addition to internal standards added during the extraction step. For separation and metabolite identification, a Waters ACQUITY ultra-performance liquid chromatography (UPLC) and a Thermo Scientific Q-Exactive high resolution/accurate mass spectrometer interfaced with a heated electrospray ionization (HESI-II) source and Orbitrap mass

analyzer were used. Raw data was extracted, peak-identified and quality controlled, and peaks identified by ma a company plasma sample tching with the Metabolon library of authenticated standards based on retention time index, mass to charge ratio, and chromatographic and tandem mass spectrometry data. The dataset of this study included 879 identified compounds. Raw data were displayed as intensity area under the curve (AUC) of ion measurements from the mass spectrometry. A detailed description of the extraction and analysis method has been previously published (Ford et al, 2020).

NAD$^+$:NADH cycling assay

NAD$^+$ and NADH was measured using a modified enzymatic cycling assay based on (Graeff & Lee, 2002). Briefly, frozen kidney tissue samples for NAD$^+$ and NADH measurements were homogenized in extraction buffer at 10 mg tissue to 100 µL buffer. For NAD$^+$ extraction, ice cold 0.6 M perchloric acid (Millipore Sigma) was used, while for NADH measurements, tissues were extracted using 0.25 M potassium hydroxide (Millipore Sigma) in 50% alcohol. The samples were centrifuged (Eppendorf 5417R at 14,000 rpm, equivalent to 18,188 g, for 10 min), and supernatants diluted 1:100 (NAD) or 1:50 (NADH) in 100 mM Na$_2$HPO$_4$ buffer, and pH adjusted to 8. Subsequently, 5 µL of extract or standard was added to 95 µL of cycling mix (0.1% Bovine serum albumin faction V, heat shock, fatty acid free (Roche, cat. #03117405001), 2% EtOH, 10 mM nicotinamide (Millipore Sigma, cat. #72345), 10 µM flavin mononucleotide (Sigma, cat. #F6750), 20 µM resazurin (Millipore Sigma, cat. #R7017), 10 µg × mL^{-1} diaphorase (Millipore Sigma, cat. #5540), 100 µg × mL^{-1} Alcohol dehydrogenase (Millipore Sigma, cat. #A3263) in 100 mM Na$_2$HPO$_4$ buffer, and pH adjusted to 8. The reaction fluorescence was read at emission of 590 nM with an excitation of 530/525 nM (Synergy H1, BioTek Winooski, VT). NAD and NADH standards serially diluted from stocks purchased from Roche (cat. #10004634103 and #10004634103, respectively).

Data analysis

Data were analyzed using MS Excel 16.24 and GraphPad Prism 8.2. We determined differences in metabolite intensity AUC measurements between individual conditions based upon ≥ 2.5-fold differences in average metabolite ion count values and statistical significance, determined by multiple t-tests with a False discovery rate (Q) of 1% (two-stage step-up method of Benjamini, Krieger and Yekutieli). Correlation analysis was conducted using Pearson's correlation. For data display into heatmaps, the peak values were normalized to the average of the CTR or NK condition and $\log_2$ transformed. For comparison of single metabolites, we used one-way ANOVA.

Supplementary Figures and Tables

Figure S1: Analysis strategy and assessment of BALB/c versus C57BL/6 dependent changes

A Step1: Determine differences, multiple t-test with FDR (Q): 1%, 2.5-fold difference (higher or lower)

decrease *increase*
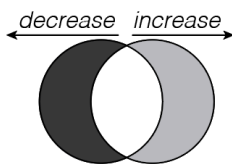
B Step 2: Assessment of experimental comparisons

ACR vs NK
transplant rejection

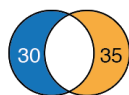IRI vs CTR
ischemia reperfusion injury
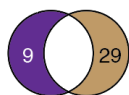
CTR vs NK
potential differences of BALB/c vs. C57BL/6 but could be confounded by circulating metabolites from rejection or IRI

C Step 3: Comparison of C67BL/6 vs. BALB/c conditions

3-methylcrotonylglycine, citramalate, 1-methylhistidine, 2S,3R-dihydroxybutyrate, butenoylglycine, isovalerylglycine, 3-methylhistidine

*stachydrine
N,N-dimethylalanine
N4-acetylcytidine
5,6-dihydrouridine*

↓ACR 145

↑CTR-NK 19

7

4

↓CTR-NK 4

3

1

42 ↑ACR
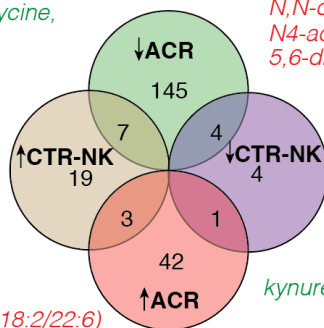
*2-hydroxyglutarate
N-acetylmethionine sulfoxide
linoleoyl-docosahexaenoyl-glycerol (18:2/22:6)*

kynurenine

Analysis of metabolomic data. (**A**) Differences between the conditions outlined in Fig. 1A were calculated using false discovery rate (FDR) adjusted multiple t-tests and a ≥ 2.5-fold differences in average metabolite intensity area under the curve (AUC) measurements. (**B**) Since the transplant condition required a full MHC-mismatch to facilitate acute cellular rejection (ACR), the native kidney (NK) is from a different mouse strain (BALB/c) than the donor kidney (C57BL/6), which in turn shares the same background as the ischemia-reperfusion injury (IRI) and non-ischemic contralateral control (CTR) kidneys (both C57BL/6). Differences between the control conditions (CTR vs. NK) may point

towards baseline metabolite differences between BALB/c and C57BL/6 strains, but could also be affected by circulating metabolites from the IRI (CTR) or ACR (NK) conditions. (**C**) Venn diagrams comparing altered metabolites between ACR and NK, as well as CTR and NK. Metabolites in red show concordant C57BL/6 > BALB/c (left lower corner) and C57BL/6 < BALB/c (right upper corner) metabolites, overlapping between both BALB/c and C57BL/6 pairings. Metabolites shown in green are discordant between both C57BL/6 and BALB/c pairings, and thus, likely independent from different mouse backgrounds.

Figure S2: Additional lysine catabolism metabolites including succinate
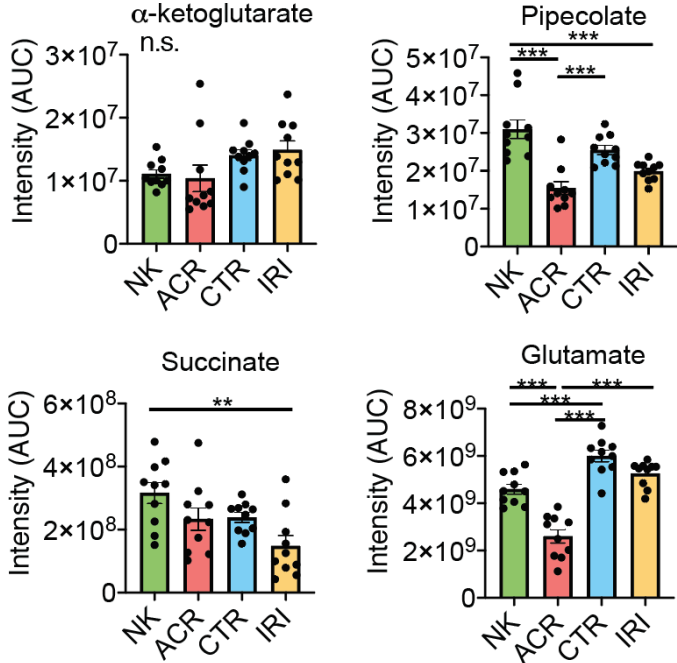
Intensity area under the curve (AUC) of metabolites associated with lysine catabolism, see Fig. 1D, E (unpaired ANOVA). *, **, and *** indicate $p<0.05$, $p<0.01$, and $p<0.001$, respectively. Data shown as mean ± SEM.

Figure S3: No methylmalonic acidemia phenotype in ACR
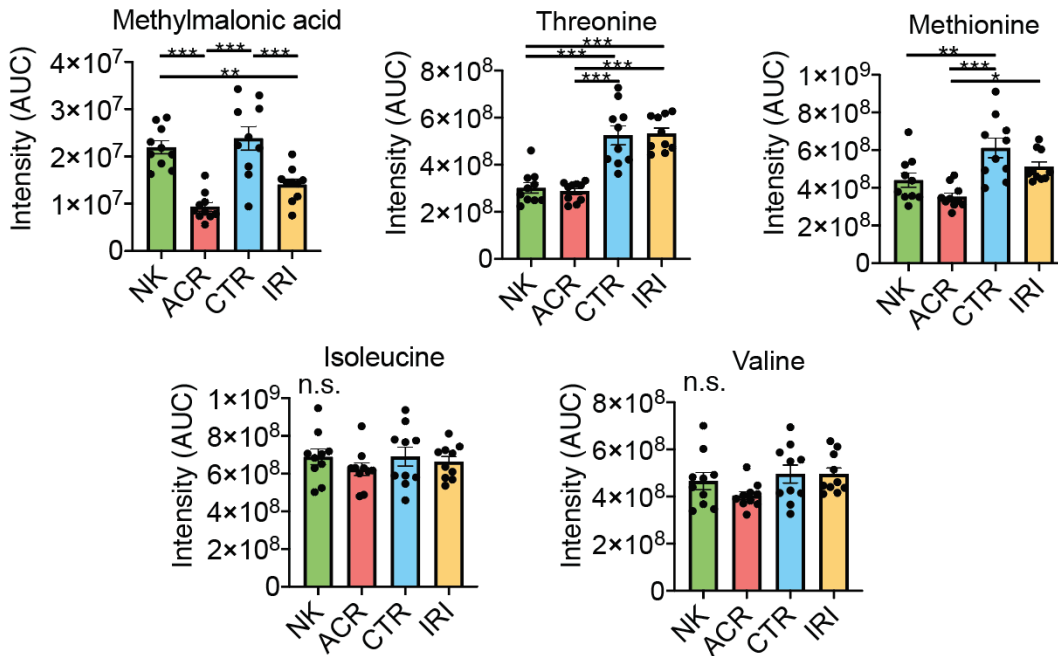
Intensity area under the curve (AUC) of methylmalonic acid and methylmalonic acidemia-associated amino acids do not show accumulation, inconsistent with a potential impairment of methylmalonyl-CoA mutase activity (unpaired ANOVA). *, **, and *** indicate $p<0.05$, $p<0.01$, and $p<0.001$, respectively. Data shown as mean ± SEM.

Figure S4: Tryptophan and kynurenine catabolism metabolites
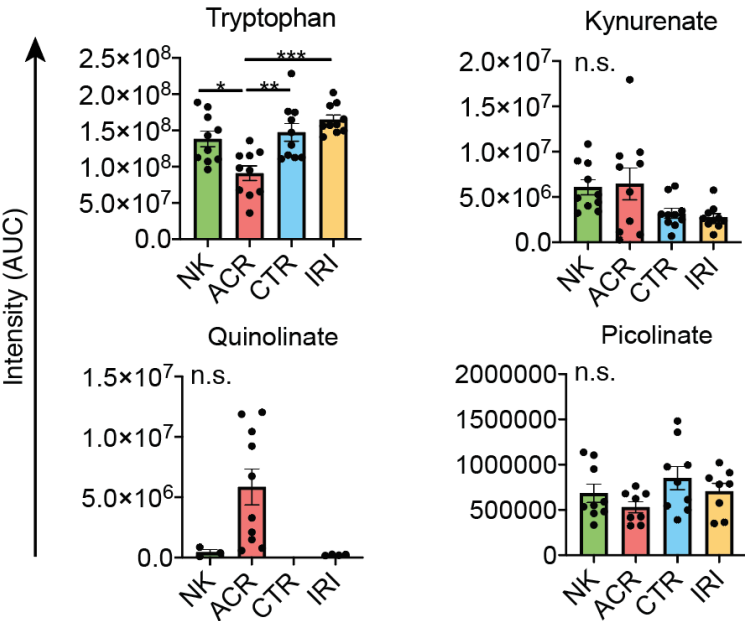
Intensity area under the curve (AUC) of tryptophan and kynurenine catabolism metabolites (unpaired ANOVA). *, **, and *** indicate p<0.05, p<0.01, and p<0.001, respectively. Data shown as mean ± SEM.

Table S1: Metabolites increased in ACR vs. NK

Name	P value	Mean NK	Mean ACR	q value	Fold ACR/NK
itaconate	0.000006	19384912	669188624	0.000038	34.52
kynurenine	0.000647	3848528	77275192	0.00174	20.08
2'-deoxycytidine	0.000153	907348	10243226	0.000537	11.29
glycosyl-N-palmitoyl-sphingosine (d18:1/16:0)	0.000432	160449	1752797	0.001255	10.92
12-HHTrE	0.000002	261289	2346230	0.000012	8.98
5-methyluridine (ribothymidine)	0.000546	85220	722089	0.00154	8.47
N-acetylaspartate (NAA)	<0.000001	1179583	9919916	0.000002	8.41
N-acetylneuraminate	0.00016	1244161	8684032	0.000556	6.98
2'-deoxycytidine 5'-monophosphate	<0.000001	129372	756277	<0.000001	5.85
palmitate (16:0)	0.00287	298251902	1679192278	0.005995	5.63
oxalate (ethanedioate)	<0.000001	3127525	17384621	<0.000001	5.56
linoleoyl-docosahexaenoyl-glycerol (18:2/22:6) [1]	0.0002	106245	577243	0.000679	5.43
palmitoyl-oleoyl-glycerol (16:0/18:1) [2]	0.00479	230361	1223528	0.009151	5.31
N-oleoylglycine	0.000015	197766	1016016	0.000081	5.14
arachidonoyl ethanolamide	0.002286	266140	1322478	0.004971	4.97
6-keto prostaglandin F1alpha	0.000287	242741	1177731	0.000927	4.85
N6,N6-dimethyllysine	0.003731	1966859	9068191	0.007544	4.61
ornithine	<0.000001	38572922	173683250	0.000002	4.50
indolepropionylglycine	0.000048	216370	925351	0.000205	4.28
N-glycolylneuraminate	0.000038	16775608	70864704	0.000174	4.22
dopamine 4-sulfate	0.000005	124607	522189	0.000033	4.19
2-hydroxyglutarate	0.000002	551945	2212888	0.000016	4.01
N-acetyl-beta-alanine	0.000005	532470	2133058	0.00003	4.01
alanylleucine	0.00491	153821	608192	0.00935	3.95
orotate	0.000118	6771345	26234454	0.000436	3.87
hexadecadienoate (16:2n6)	0.002599	506179	1874722	0.005509	3.70
(16 or 17)-methylstearate (a19:0 or i19:0)	0.001247	357272	1318611	0.002993	3.69
N-acetylphenylalanine	0.000051	144924	499962	0.000213	3.45
2-hydroxyarachidate	0.001955	114066	393031	0.004334	3.45
linoleoyl-docosahexaenoyl-glycerol (18:2/22:6) [2]	0.000422	1419081	4875575	0.001232	3.44
linoleoyl-arachidonoyl-glycerol (18:2/20:4) [2]	0.00005	817282	2722118	0.000209	3.33
heme	0.000271	1041853	3398697	0.000884	3.26
N-acetylmethionine sulfoxide	0.000843	640526	2052496	0.002187	3.20
1-linoleoyl-GPS (18:2)	0.000004	1329753	4174747	0.000028	3.14
urate	0.003276	7310696	22871356	0.006795	3.13

2'-deoxyinosine	0.001602	95241	289932	0.003672	3.04
N-acetyltaurine	0.000035	1640370	4922443	0.000164	3.00
margarate (17:0)	0.003992	8446656	24186506	0.007903	2.86
cytidine 5'-monophospho-N-acetylneuraminic acid	0.000151	324410	926702	0.000534	2.86
2'-deoxyadenosine	<0.000001	451139	1243208	0.000003	2.76
stearate (18:0)	0.004177	570596989	1561813536	0.008211	2.74
1-(1-enyl-stearoyl)-GPE (P-18:0)	0.003848	2282016	6087308	0.007733	2.67
1-myristoyl-2-palmitoyl-GPC (14:0/16:0)	0.000774	3218176	8527971	0.002025	2.65
2-hydroxybehenate	0.002297	293921	752708	0.004977	2.56
sedoheptulose-7-phosphate	0.001808	1389608	3500697	0.004054	2.52
stearoylcarnitine (C18)	0.001102	29137708	73022718	0.002688	2.51

Table S2: Metabolites decreased in ACR vs. NK

Name	P value	Mean NK	Mean ACR	q value	Fold NK/ACR
gamma-glutamylisoleucine	<0.000001	2694793	1075255	0.000004	2.51
arabonate/xylonate	0.000281	8660153	3439363	0.000911	2.52
cystathionine	0.000918	2139387	848923	0.002318	2.52
spermine	0.003393	513126	203560	0.006958	2.52
1-stearoyl-2-arachidonoyl-GPS (18:0/20:4)	0.000027	47774379	18899861	0.00013	2.53
gamma-carboxyglutamate	0.000097	13848773	5476052	0.000362	2.53
xanthurenate	0.000677	1177314	464605	0.001805	2.53
sphingomyelin (d18:1/22:1, d18:2/22:0, d16:1/24:1)	0.000668	5716762	2253658	0.001787	2.54
N1-methylinosine	0.003332	1867328	730557	0.006886	2.56
3-methylcrotonylglycine	0.002312	606496	234375	0.004991	2.59
stearoyl-docosahexaenoyl-glycerol (18:0/22:6) [2]	0.000016	1195513	459051	0.000084	2.60
butenoylglycine	0.000877	372437	142271	0.002242	2.62
lignoceroyl sphingomyelin (d18:1/24:0)	0.000006	4022102	1529655	0.000038	2.63
choline	<0.000001	2196201638	833339331	<0.000001	2.64
mannonate	0.000256	6159384	2322413	0.000848	2.65
5-methyltetrahydrofolate (5MeTHF)	0.002706	299563	112369	0.005674	2.67
1-oleoyl-GPE (18:1)	0.000355	7652434	2865398	0.001071	2.67
uridine	<0.000001	107247010	40009484	<0.000001	2.68
5,6-dihydrouridine	0.000116	3038055	1132474	0.000431	2.68
N4-acetylcytidine	0.000121	3426002	1268051	0.000438	2.70
acetyl CoA	0.000922	173532	64227	0.002319	2.70
1-palmitoyl-GPS (16:0)	0.003953	1840509	680663	0.007863	2.70
1-stearoyl-2-arachidonoyl-GPE (18:0/20:4)	0.000006	48012055	17721899	0.000038	2.71
arabitol/xylitol	0.000045	5581373	2040755	0.000198	2.73
1-oleoyl-2-docosahexaenoyl-GPC (18:1/22:6)	<0.000001	12214911	4388933	0.000005	2.78
phosphoenolpyruvate (PEP)	0.00013	3934621	1406197	0.000471	2.80
valylleucine	0.000618	365778	129793	0.001702	2.82
pro-hydroxy-pro	0.000234	19635562	6954233	0.00078	2.82
gamma-glutamylglycine	0.000047	48034598	16527961	0.000205	2.91
urea	0.000022	226869821	77839900	0.000111	2.91
pterin	0.000088	2588399	883592	0.000338	2.93
pantothenate	<0.000001	41235457	14029609	0.000001	2.94
galactose 1-phosphate	<0.000001	1760597	582269	0.000004	3.02
N-acetylasparagine	0.000119	1844634	607289	0.000436	3.04
ribulonate/xylulonate/lyxonate	0.000056	9266517	3013544	0.000227	3.07

stachydrine	0.000004	61712795	19943418	0.000027	3.09
pyruvate	<0.000001	31951508	10187843	<0.000001	3.14
allantoin	0.000119	79681421	25258528	0.000436	3.15
maleate	0.000015	6839767	2147828	0.00008	3.18
2S,3R-dihydroxybutyrate	0.001429	5878621	1838847	0.003332	3.20
argininosuccinate	0.000093	8202226	2557327	0.000352	3.21
inosine	<0.000001	339644010	104422939	<0.000001	3.25
S-1-pyrroline-5-carboxylate	<0.000001	3683983	1126229	<0.000001	3.27
propionylcarnitine (C3)	0.000132	69363487	20999566	0.000473	3.30
7-methylguanine	0.004784	644036	194406	0.009151	3.31
inositol 1-phosphate (I1P)	0.000046	1182183	355403	0.000201	3.33
1-oleoyl-2-linoleoyl-GPE (18:1/18:2)	0.00004	4942520	1482765	0.000179	3.33
2-aminophenol sulfate	0.000639	1033509	306962	0.001727	3.37
thiamin diphosphate	0.000024	612081	176445	0.000119	3.47
alpha-ketoglutaramate	0.000079	6039420	1735746	0.000313	3.48
N-methylproline	0.000011	5870022	1680672	0.000064	3.49
N-methyl-GABA	<0.000001	70458342	20018210	0.000004	3.52
arginine	0.000003	306433890	86699541	0.000019	3.53
ethylmalonate	<0.000001	25761825	7284003	0.000002	3.54
trimethylamine N-oxide	0.000496	68097618	18971863	0.001418	3.59
docosahexaenoylcarnitine (C22:6)	0.003381	5109960	1418844	0.006958	3.60
N2,N5-diacetylornithine	0.00139	348769	96830	0.003256	3.60
N-acetylcitrulline	0.000346	509570	141346	0.001064	3.61
S-methylcysteine	<0.000001	770358	209948	0.000002	3.67
1-palmitoyl-2-docosahexaenoyl-GPE (16:0/22:6)	<0.000001	11978288	3247257	0.000003	3.69
N-acetyl-glucosamine 1-phosphate	<0.000001	4372350	1171467	<0.000001	3.73
histidine methyl ester	0.000003	3154043	844849	0.000022	3.73
1-linoleoyl-2-arachidonoyl-GPE (18:2/20:4)	<0.000001	3239719	853572	<0.000001	3.80
butyrylglycine	0.000019	2725547	714171	0.000098	3.82
cysteinylglycine	0.000048	3172558	826898	0.000205	3.84
1-palmitoyl-2-oleoyl-GPG (16:0/18:1)	0.000017	2518626	636980	0.000089	3.95
corticosterone	0.000628	818049	204715	0.00172	4.00
sphingomyelin (d18:2/14:0, d18:1/14:1)	0.001259	332853	79720	0.002997	4.18
guanosine	<0.000001	31080036	7404041	<0.000001	4.20
4-hydroxyhippurate	0.00088	1056416	251449	0.002243	4.20
trigonelline (N'-methylnicotinate)	<0.000001	252830574	59994622	0.000002	4.21
genistein sulfate	0.004185	3644235	851211	0.008211	4.28
4-methylcatechol sulfate	0.001207	292701	67702	0.002921	4.32

oleoylcholine	0.000001	539649	124550	0.000011	4.33
2R,3R-dihydroxybutyrate	0.000002	21136713	4877837	0.000012	4.33
2-methylbutyrylglycine	0.000002	340049	78228	0.000014	4.35
2-(4-hydroxyphenyl)propionate	0.001627	1064660	244463	0.003706	4.36
sphingomyelin (d18:2/16:0, d18:1/16:1)	0.000004	84787999	19001327	0.000028	4.46
guanidinoacetate	<0.000001	590345926	129920993	<0.000001	4.54
N-octanoylglycine	0.003934	570458	124282	0.007852	4.59
pyridoxal	<0.000001	29087010	6328376	<0.000001	4.60
propionylglycine	0.0003	327695	69320	0.000962	4.73
citramalate	0.000037	11747439	2471997	0.000168	4.75
3-aminoisobutyrate	<0.000001	3990246	833687	0.000002	4.79
S-(3-hydroxypropyl)mercapturic acid (HPMA)	0.000639	374028	75485	0.001727	4.95
1-palmitoyl-2-arachidonoyl-GPE (16:0/20:4)	<0.000001	25682288	5141384	0.000002	5.00
pyridoxate	0.000206	4398976	876009	0.000695	5.02
adipoylcarnitine (C6-DC)	0.000012	11554868	2239761	0.000064	5.16
equol glucuronide	0.000054	2695696	514013	0.000223	5.24
3-methylglutaconate	0.000004	711794	134235	0.000028	5.30
linoleoylcholine	0.000173	411364	77115	0.000595	5.33
leucylglutamine	0.001213	1034449	191819	0.002923	5.39
4-acetylphenol sulfate	0.000033	381018	70385	0.000154	5.41
4-hydroxycinnamate sulfate	0.001975	1555686	286113	0.004362	5.44
nicotinate ribonucleoside	0.00002	4463659	804653	0.000106	5.55
glycerol	<0.000001	10580949	1858814	<0.000001	5.69
betaine aldehyde	0.000158	13425541	2346324	0.000554	5.72
thioproline	<0.000001	305937574	53339188	<0.000001	5.74
thiamin monophosphate	<0.000001	703260	122428	<0.000001	5.74
malonylcarnitine	<0.000001	6947508	1209320	<0.000001	5.74
2,3-dihydroxy-5-methylthio-4-pentenoate (DMTPA)	0.000007	752120	128864	0.000044	5.84
pyroglutamine	0.000001	17471528	2990040	0.000009	5.84
galactonate	0.002166	72705969	12191282	0.004747	5.96
S-adenosylhomocysteine (SAH)	<0.000001	4810771	795308	<0.000001	6.05
isovalerylglycine	<0.000001	7283616	1170676	<0.000001	6.22
N6-succinyladenosine	0.000024	11039185	1671814	0.000118	6.60
thiamin (Vitamin B1)	<0.000001	17142930	2570531	<0.000001	6.67
3-hydroxycinnamate sulfate	0.001475	366788	54952	0.003414	6.67
1-oleoyl-2-docosahexaenoyl-GPE (18:1/22:6)	<0.000001	7801801	1161770	0.000002	6.72
flavin adenine dinucleotide (FAD)	<0.000001	4871494	713706	<0.000001	6.83
cysteine sulfinic acid	<0.000001	5946501	861239	<0.000001	6.90

1-palmitoyl-2-docosahexaenoyl-GPC (16:0/22:6)	<0.000001	209891678	29380771	<0.000001	7.14
N,N-dimethylalanine	0.0005	107072261	14544932	0.001424	7.36
guanidinosuccinate	0.000066	2301955	309572	0.000265	7.44
isocaproylglycine	0.003402	659116	88306	0.006958	7.46
riboflavin (Vitamin B2)	<0.000001	4247299	555013	<0.000001	7.65
methylphosphate	<0.000001	330750709	43114023	<0.000001	7.67
cystine	<0.000001	280331382	36123311	<0.000001	7.76
1-oleoyl-2-arachidonoyl-GPE (18:1/20:4)	<0.000001	31164720	4014882	0.000001	7.76
1-methylhistamine	0.000079	63806391	8119552	0.000313	7.86
succinylcarnitine (C4-DC)	0.000004	4464838	566817	0.000029	7.88
ribose	<0.000001	37794680	4668602	<0.000001	8.10
ascorbic acid 2-sulfate	0.000002	25972139	3205740	0.000012	8.10
valylglutamine	0.001874	875943	105688	0.004185	8.29
3-(3-hydroxyphenyl)propionate sulfate	0.000011	22458167	2694634	0.000064	8.33
3-methylhistidine	0.000002	55591646	6498748	0.000017	8.55
S-carboxymethyl-L-cysteine	<0.000001	1363630	151097	<0.000001	9.02
formiminoglutamate	<0.000001	32238986	3013596	<0.000001	10.70
hydroxyasparagine	<0.000001	2627898	239974	0.000008	10.95
pyridoxamine phosphate	<0.000001	3357600	304860	0.000002	11.01
flavin mononucleotide (FMN)	<0.000001	1194504	106667	<0.000001	11.20
cyano-alanine	0.000015	1860839	164502	0.000081	11.31
glycine conjugate of C6H10O2 (2)	0.000328	938764	81193	0.001031	11.56
1-methylhistidine	<0.000001	23392558	1855429	0.000003	12.61
hydroxy-N6,N6,N6-trimethyllysine	<0.000001	2502158	196681	0.000001	12.72
ribitol	<0.000001	10521026	815383	<0.000001	12.90
glycerol 3-phosphate	<0.000001	100609346	7696170	<0.000001	13.07
glutarylcarnitine (C5-DC)	0.000002	8183967	623231	0.000018	13.13
2,8-quinolinediol sulfate	0.000007	2253889	163766	0.00004	13.76
gulonate	<0.000001	304910213	21533482	0.000001	14.16
N-acetylcysteine	<0.000001	3795154	261374	0.000001	14.52
cysteine	<0.000001	170653925	10859823	0.000002	15.71
N6-carboxymethyllysine	<0.000001	17926150	926920	0.000003	19.34
anserine	0.000001	46909979	2362674	0.000012	19.85
N6-carboxyethyllysine	<0.000001	7424988	331116	0.000001	22.42
nicotinamide adenine dinucleotide (NAD+)	0.00009	6510248	281757	0.00034	23.11
nicotinamide riboside	<0.000001	48195509	2082945	<0.000001	23.14
nicotinamide ribonucleotide (NMN)	<0.000001	8129543	347459	0.000004	23.40
5-hydroxylysine	<0.000001	16388606	699442	0.000008	23.43

4-hydroxyglutamate	0.000435	7776085	294291	0.001257	26.42
11-dehydrocorticosterone	0.004697	1239767	44533	0.009078	27.84
pimeloylcarnitine/3-methyladipoylcarnitine (C7-DC)	0.000362	9941922	293620	0.001083	33.86
carnosine	0.000031	135309276	3490253	0.000144	38.77
lanthionine	<0.000001	20484449	331492	<0.000001	61.79
pyridoxamine	<0.000001	64781883	1039153	<0.000001	62.34
glucuronate	<0.000001	100017289	1493890	<0.000001	66.95

Table S3: Metabolites increased in IRI vs. CTR

Name	P value	Mean IRI	Mean CTR	q value	Fold IRI/CTR
1-docosahexaenoylglycerol (22:6)	0.000806	51204704	20211974	0.003075	2.53
eicosenoate (20:1)	0.000028	35326915	13865770	0.000204	2.55
erucate (22:1n9)	0.000032	5179960	1993414	0.000222	2.60
dihomo-linoleate (20:2n6)	0.000105	28904068	11102160	0.000557	2.60
(3'-5')-adenylyladenosine	0.002484	406276	155940	0.007178	2.61
2-hydroxybehenate	<0.000001	539575	204591	0.000003	2.64
myristoleate (14:1n5)	0.000076	5781816	2185110	0.000423	2.65
3-hydroxyarachidate	0.000032	92747	34910	0.000223	2.66
beta-citrylglutamate	<0.000001	760086	285673	<0.000001	2.66
tetradecadienoate (14:2)	0.000041	2895651	1084726	0.000269	2.67
eicosapentaenoate (EPA; 20:5n3)	0.000866	12446624	4644592	0.003243	2.68
docosadienoate (22:2n6)	0.000061	2978987	1047887	0.000369	2.84
putrescine	<0.000001	4385982	1525792	<0.000001	2.87
fructose	0.001079	8743981	2945749	0.003758	2.97
2'-deoxycytidine	0.000023	2308114	743822	0.000171	3.10
indoleacetate	0.000217	114215	35302	0.001027	3.24
maltose	0.000562	2851171	866309	0.002307	3.29
2-hydroxyarachidate	<0.000001	284958	85308	<0.000001	3.34
3-formylindole	0.001791	1458159	427503	0.005495	3.41
1-linoleoyl-GPG (18:2)	<0.000001	10484109	2977981	<0.000001	3.52
1-stearoyl-GPG (18:0)	<0.000001	1158288	327592	<0.000001	3.54
2-aminoadipate	0.000002	19904275	5599949	0.000022	3.55
orotate	<0.000001	22102733	6009434	<0.000001	3.68
2-hydroxynervonate	0.000001	166795	45082	0.000017	3.70
4-hydroxyphenylpyruvate	0.000084	741755	198204	0.000464	3.74
nervonate (24:1n9)	<0.000001	3079658	797004	<0.000001	3.86
itaconate	<0.000001	16880428	4163802	0.000002	4.05
3-indoleglyoxylic acid	0.000709	93387	21559	0.002753	4.33
heme	0.000253	1001390	122977	0.001148	8.14
saccharopine	<0.000001	21589360	1728235	<0.000001	12.49

Table S4: Metabolites decreased in IRI vs. CTR

Name	P value	Mean IRI	Mean CTR	q value	Fold CTR/IRI
hydroxyasparagine	0.000018	192110	2341591	0.000138	12.19
1-methylhistidine	<0.000001	13824875	89722793	0.000002	6.49
formiminoglutamate	0.000001	10974449	62556392	0.000014	5.70
cyano-alanine	<0.000001	537814	3003157	<0.000001	5.58
N6-carboxymethyllysine	<0.000001	3474691	17827582	<0.000001	5.13
homocitrate	0.000242	1856133	9197317	0.001105	4.96
3-methylhistidine	<0.000001	32331394	156596127	<0.000001	4.84
cysteine sulfinic acid	<0.000001	3195651	14434586	0.00001	4.52
N6-carboxyethyllysine	<0.000001	1053502	4513293	0.000011	4.28
pyroglutamine	<0.000001	2813355	11926171	<0.000001	4.24
pantetheine	0.000265	64912	248135	0.001186	3.82
malonylcarnitine	<0.000001	1743421	6296305	<0.000001	3.61
nicotinamide riboside	<0.000001	24115074	82131411	<0.000001	3.41
N-octanoylglycine	0.000014	199178	676942	0.000111	3.40
phosphopantetheine	0.000093	349001	1172724	0.000502	3.36
1-methyladenine	0.000652	103597	347409	0.002562	3.35
N-acetyl-1-methylhistidine	<0.000001	1102530	3673936	0.000003	3.33
5-hydroxylysine	0.000001	6179205	19306160	0.000017	3.12
1-methyl-4-imidazoleacetate	0.00016	11880863	36247737	0.000791	3.05
1-methylhistamine	0.000013	13788474	40832609	0.000109	2.96
1-ribosyl-imidazoleacetate	0.000004	1791115	5152320	0.000039	2.88
S-methylcysteine	<0.000001	445933	1280745	<0.000001	2.87
ribitol	0.00002	6520416	18370520	0.000149	2.82
tricarballylate	0.000082	13553423	38114331	0.000455	2.81
lanthionine	<0.000001	9778598	27437416	<0.000001	2.81
O-sulfo-L-tyrosine	0.000064	320149	893659	0.000376	2.79
thiamin (Vitamin B1)	0.000003	11149117	30830764	0.000032	2.77
guanidinosuccinate	0.001049	461730	1264501	0.003695	2.74
dopamine 3-O-sulfate	0.00011	29475	78472	0.000579	2.66
ectoine	0.000002	926735	2419214	0.000026	2.61
succinylcarnitine (C4-DC)	0.000041	2569408	6620681	0.000269	2.58
pentose acid	0.001739	615933	1586036	0.005413	2.58
adenine	<0.000001	14607810	37328808	0.000004	2.56
citramalate	0.00308	15115699	38599785	0.008556	2.55
2,3-dihydroxy-5-methylthio-4-pentenoate (DMTPA)	0.000062	291664	729727	0.000375	2.50

Supplementary References

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