UHPLC/MS based large-scale targeted metabolomics method for multiple-biological matrix assay

Xialin Luo[1], Aihua Zhang[2], Xijun Wang[2]*, and Haitao Lu[1]*

[1]Key Laboratory of Systems Biomedicine (Ministry of Education), Shanghai Center for Systems Biomedicine, Shanghai Jiao Tong University, Shanghai 200240, China

[2]National Chinmedomics Research Center, Sino-America Chinmedomics Technology Collaboration Center, National TCM Key Laboratory of Serum Pharmacochemistry, Laboratory of Metabolomics, Department of Pharmaceutical Analysis, Heilongjiang University of Chinese Medicine, Heping Road 24, Harbin, China

Corresponding authors. Prof. Haitao Lu, and Prof. Xijun Wang

haitao.lu@sjtu.edu.cn and xijunwangls@126.com

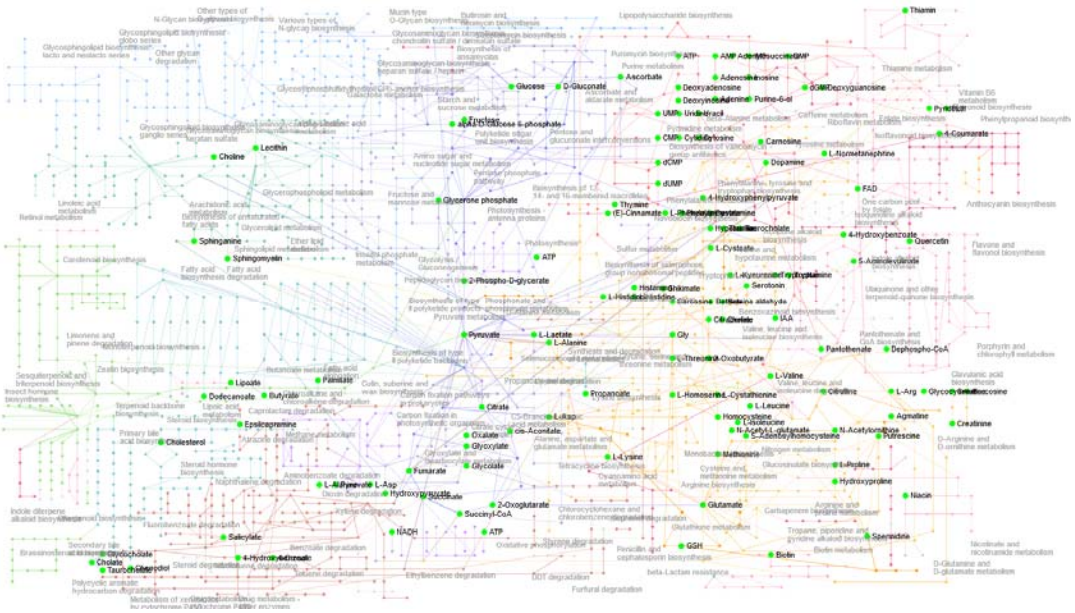
Introduction

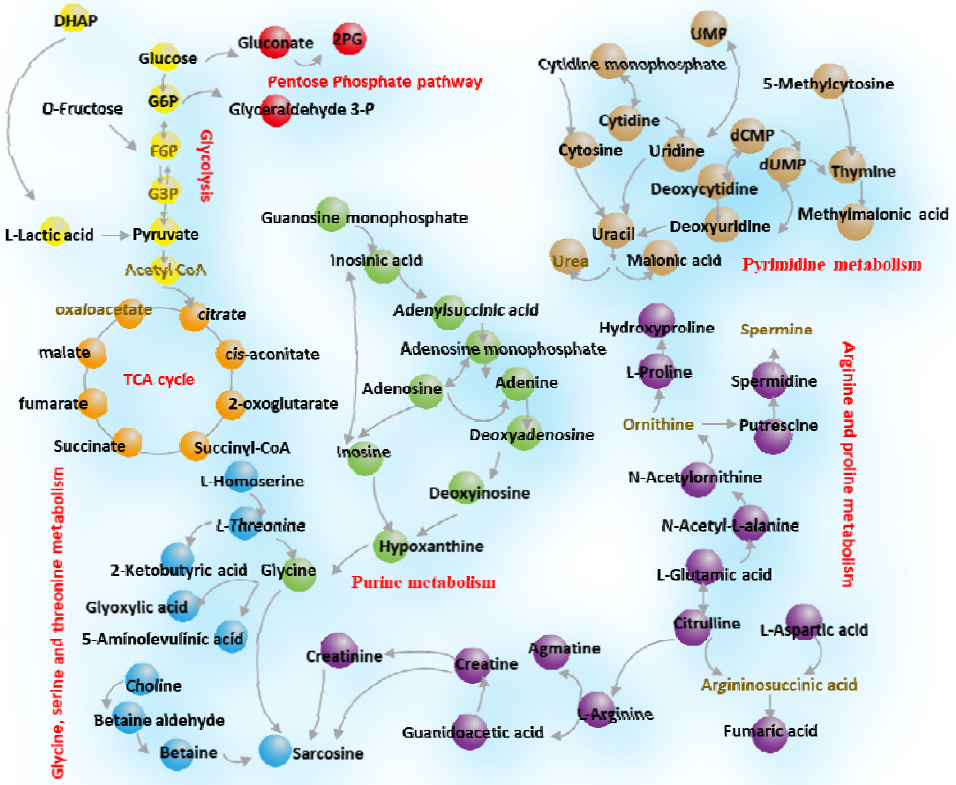
Metabolism is characterized by a series of essential life-sustaining processes in all organisms by providing the living cells with necessary nutrients and energy, which enable the cells to grow, differentiate and functioning. Characterizing altered metabolism underlying a diversity of biochemical events and/or processes, we have the capability to solve the key problems in different niches as biomedicine, bioengineering, agriculture and environment[1-3]. In the late 1990s, systems biology driven metabolomics method was first to be proposed to provide a comprehensive approach to precisely investigate metabolism[4,5], via global and quantitative analysis of endogenous metabolites in biological systems requiring high-resolution analytical technologies[6]. Compared to NMR, chromatographic separation techniques such as gas or liquid chromatography (GC or LC) coupled to mass spectrometry (MS) has become the primary option to engage in the development of metabolomics method. Most effort has been invested by the scientific community on untargeted metabolomics methods for analyzing tissue and urine samples to capture the most comprehensive-coverage to small-molecule metabolomes[7,8]. However, untargeted metabolomics has obviously scientific limitations, particularly the precise-identification of differential metabolites is the greatest challenge that mostly impedes further decipher altered metabolism associated biological functions. To overcome this deficiency, some scientists recently attempt to develop targeted metabolomics method using the available reference compounds involved in many key-metabolic pathways, and further functional researches on regulatory genes and biosynthetic enzymes can help precisely interrogate the biochemical mechanisms underlying the altered metabolism[9,10]. Developing high-throughput LC-MS based targeted metabolomics method to large-scale analysis of the known metabolomes that is likely to be of great value to the investigation of life sciences[11,12,18].

Results and Discussion

Referring to our previous effort[18], we carefully selected approximately 212 key metabolites involved in numerous key metabolic pathways (see **Figure 1**), they

include organic acids, fatty acids, sugars, phosphate-sugars, amino acids and lipids, etc., which are highly associated with the disease progression, pathogenesis and therapeutic discovery[13-15]. Unlike some analytical methods prefer mostly to increase the number of analytes and/or shorten the analytical time, we aimed at accurately analyzing the biochemically small-molecule metabolites from differently biological matrixes to characterize the mostly affected metabolic pathways, by which we can further pursue functional experiments of the available reference compounds to delineate biochemical mechanisms underlying modified metabolic pathways. Therefore, we are pleased to argue that this targeted metabolomics method has greatly applicable potential in translational and precision medicine.




DHAP
Glucose
Gluconate
2PG
Pentose Phosphate pathway
D-Fructose
G6P
Glyceraldehyde 3-P
F6P
Glycolysis
G3P
L-Lactic acid
Pyruvate
Acetyl CoA
oxaloacetate
citrate
malate
cis-aconitate
TCA cycle
fumarate
2-oxoglutarate
Succinate
Succinyl-CoA
L-Homoserine
L-Threonine
2-Ketobutyric acid
Glycine
Glyoxylic acid
5-Aminolevulinic acid
Choline
Betaine aldehyde
Betaine
Sarcosine
Glycine, serine and threonine metabolism
Guanosine monophosphate
Inosinic acid
Adenylsuccinic acid
Adenosine monophosphate
Adenosine
Adenine
Inosine
Deoxyadenosine
Deoxyinosine
Hypoxanthine
Purine metabolism
Creatinine
Creatine
Guanidoacetic acid
Agmatine
L-Arginine
UMP
Cytidine monophosphate
5-Methylcytosine
Cytidine
Cytosine
Uridine
dCMP
dUMP
Thymine
Deoxycytidine
Deoxyuridine
Methylmalonic acid
Uracil
Urea
Malonic acid
Pyrimidine metabolism
Hydroxyproline
Spermine
L-Proline
Spermidine
Ornithine
Putrescine
N-Acetylornithine
N-Acetyl-L-alanine
L-Glutamic acid
Citrulline
L-Aspartic acid
Argininosuccinic acid
Fumaric acid
Arginine and proline metabolism

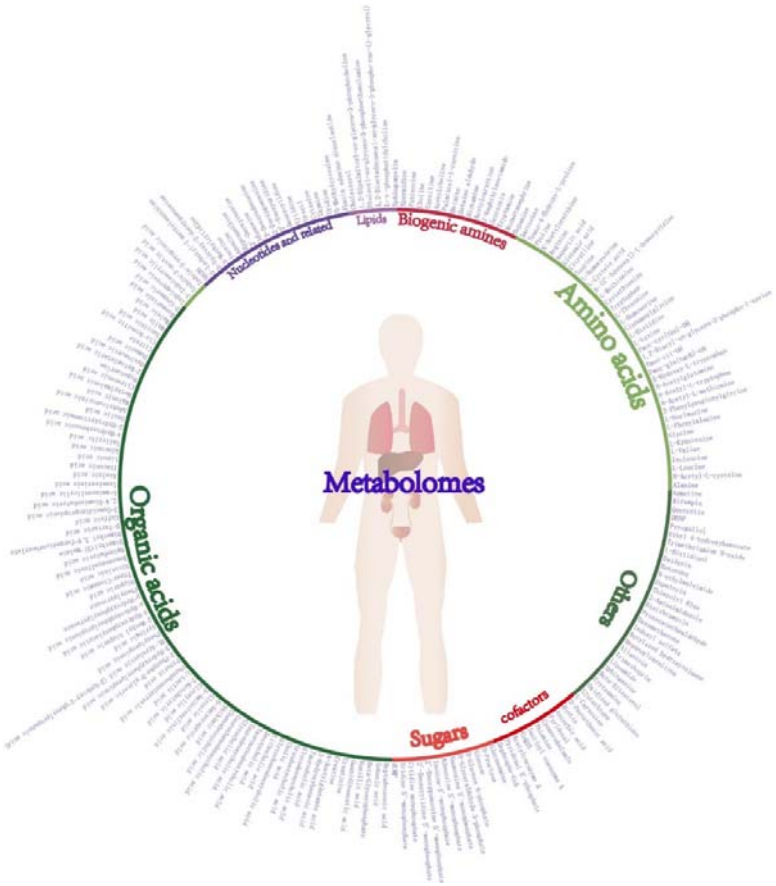


Figure 1. The key metabolic pathways associated small-molecule metabolites of interest covered by the new-developed method

This protocol for targeted metabolomics method was intensively developed to 212 known metabolites (**see Table 1**) with significantly biological functions by employing dynamic multiple reaction monitoring (DMRM) mode with ultra-performance liquid chromatography coupled with triple quadrupole mass spectrometry (UPLC-TQMS) system. TQMS is superior for its high sensitivity and specificity, lower interference as well as excellent quantitation-ability, the two-steps MS monitoring for precursor ions and product ions significantly improve the analytical selectivity and sensitivity of targeted compounds[16]. Our targeted metabolomics method by TQMS was explored and exploited to analyze diverse biological samples, such as urine, serum, cell and different rat tissues (e.g. brain, liver, heart, spleen, lung, kidney and intestine) harvested from the rats **(Figure 2)**. This method must be a useful method for precisely

analyzing targeted metabolomes present in differently biological matrixes, by which we can figure out translational medicine research to address different questions involving disease diagnosis, pathogenesis and therapeutic discovery.

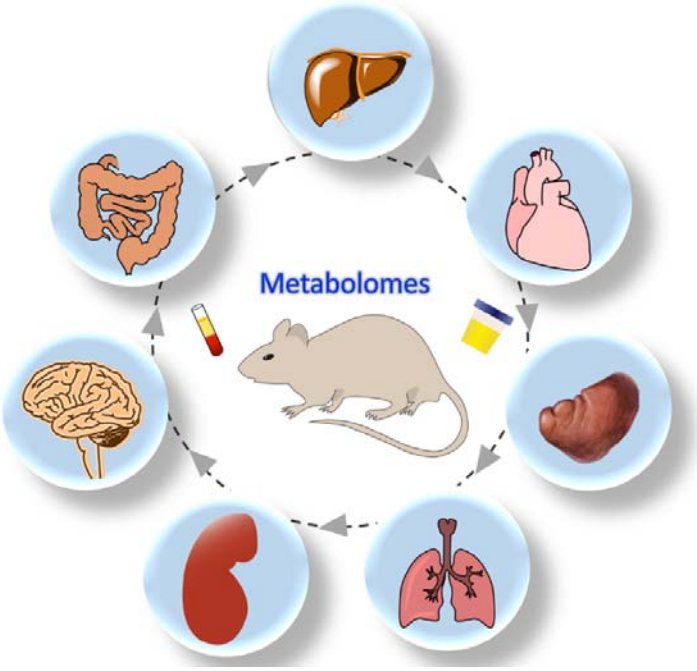


Figure 2. The new-developed method with our effort has the capacity to analyze metabolomes of interest present in a diversity of biological matrixes.

Basically, conventional MRM mode enabling targeted metabolomics method often suffers from poor sensitivity due to the dual limitations of cycle and dwell time, when come to analyze hundreds of metabolites. Our new method by DMRM mode has significantly improved the MS duty cycle time and automatically distributed the appropriate dwell time for each transition by only monitoring the targeted ions while eluted from the LC system[17]. Owing to targeted metabolites of interest covered a variety of polar and nonpolar hydrophobic metabolomes, one column-system with the defined chemistry is incapable of high-sensitivity profiling all of them, we accordingly developed two complementary liquid chromatographic approaches, using reversed-phase liquid chromatography (RPLC) for non-polar metabolomes and hydrophilic interaction liquid chromatography (HILIC) for polar metabolomes

(**Figure 3**).

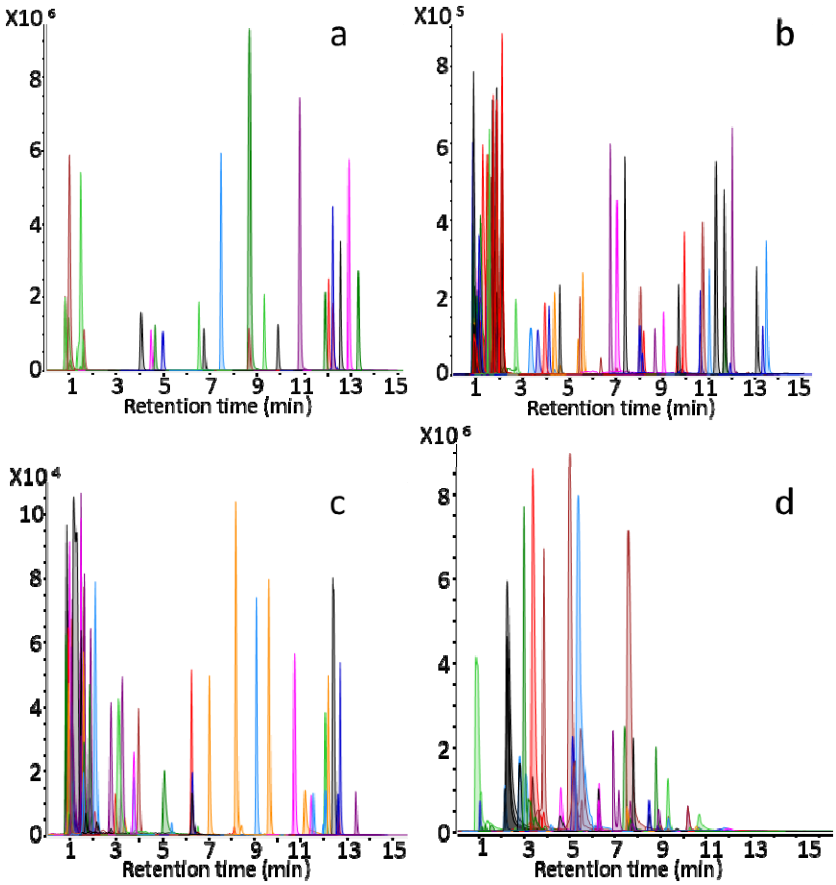


Figure 3. The typical EIC profiles for the known metabolites of interest analyzed by RPLC (abc) and HILIC columns (d), respectively.

Back to 2011, we have developed an MRM-based MS method for rapid and broad profiling of 112 hydrophilic metabolites from multiple-biological matrixes[18]. However, the old method only used a reversed-phase column without HILIC column, and the shorten reference-compounds certainly limited the number of metabolites of interests then. In addition, it's applicability to the analysis of differential biological matrixes is far inferior to the new method in this study.

To develop this applicable protocol, we have delicately optimized all the parameters involving the chromatographic separation, MS detection, sample-preparation, data analysis and visualization using the available reference-compounds, then to profile

targeted metabolites of interests in different tissues (brain, heart, liver, spleen, kidney, small intestine and lung) and body fluids (urine and plasma) collected from the rats with different dually biological treatments (**Figure 4**). It can be observed that this new method had a great capacity to stably analyze targeted metabolomes of interest present in multiple biological matrixes. Furthermore, our data revealed that metabolomes rendered tissue-specific differentiations, suggesting that the most sensitive biological samples covered the differential metabolites should be determined for the first instance when we figure out to perform metabolomics study, rather than engage in aimlessly metabolic profiling based on the regular samples such as urine and plasma. Therefore, sampling optimization should be fully considered during the diagnosis and therapeutics discovery against the different diseases, which must provide novel insights into tissue-associated disease development and therapeutics.

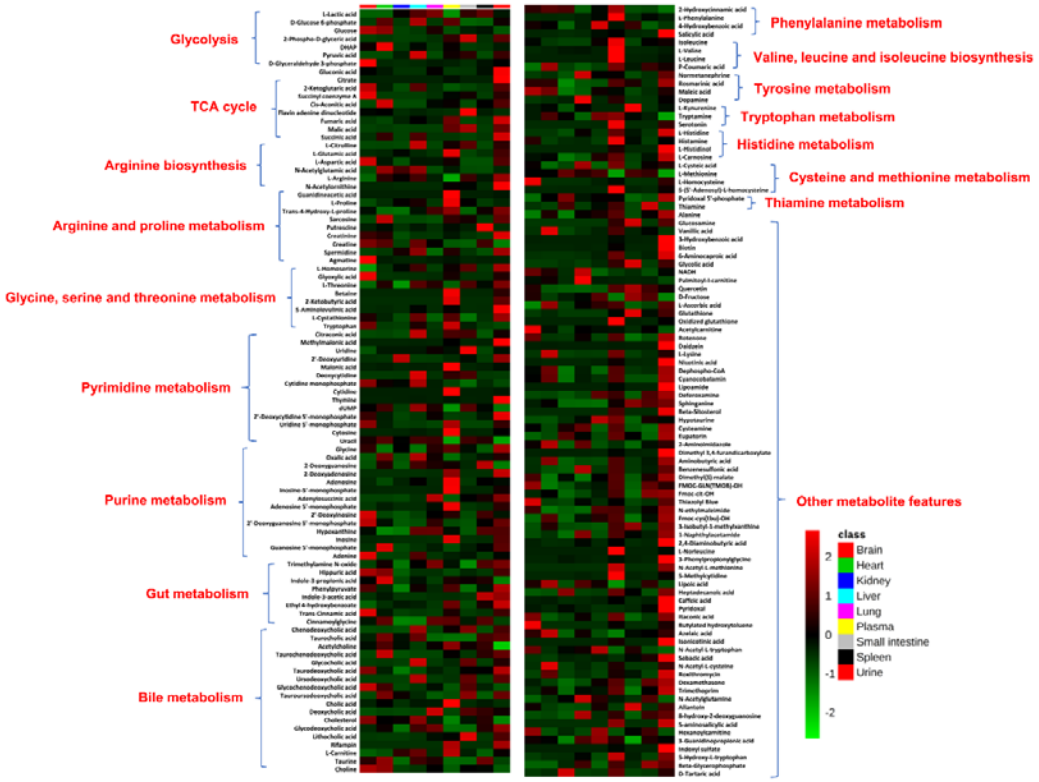


Figure 4. Heatmap overview of metabolites of interest throughout differently biological matrixes collected from the rats.

Specific metabolites are of great biochemical significance. For an example, a high

level of lactate in blood can be a sign of liver and kidney diseases even the cancer, therefore the quantification of functional metabolites shows great potential application in disease diagnosis and pathogenesis[19,20]. In our protocol, we used a set of external standards each containing at least five-levels concentration to establish the calibration curves, finally, 84 key metabolites can be absolutely quantitated by the established LC-MS method **(Table 2)**. For instance, this quantitative metabolomics method is successfully apply to quantify the concentration of 27 key metabolites in plasma, urine and different tissues of rats **(Table 3)**.

In summary, we develop a simply, greatly reliable and large-scale targeted metabolomics method that is exact coverage to more than 200 key metabolites with importantly biological functions. These metabolites involve in numerous metabolic pathways underlying different biochemical processes, including disease progression, drug treatment and genetic modifications. Facing to clinical demand, this method can also determine the absolute concentration of 84 metabolites produced by various biological matrixes, which can assist in clinical diagnosis of different diseases, by providing more information-rich data than conventional clinical chemistry assay. Herein, once this metabolomics protocol is open to the scientific community, it can be broadly applied to address a diversity of biochemical questions related to diseases and drug development,

EXPERIMENTAL SECTION

Chemicals and reagents.

The analytical standards were all purchased from Sigama-Aldrich (Shanghai, China), and dissolved in water or methanol respectively at the concentration of 1 mg/mL. HPLC grade methanol and acetonitrile were obtained from Fisher Chemical (Shanghai, China). Formic acid (HPLC-grade) was purchased from Kermel (Tianjin, China). The water we used in experiments is Milli-Q water.

UPLC-TQ MS

An UPLC system (1290 Infinity series, Agilent Technologies) coupled to a triple quadrupole mass spectrometer (Agilent 6495, Agilent Technologies) was used for more than 212 metabolites profiling. The ion source was Agilent Jet Stream ESI and the parameters were set as follows: dry gas temperature, 250 ^{o}C; dry gas flow, 16 L/min; nebulizer pressure, 20 psi; sheath gas heater, 400 ^{o}C; sheath gas flow, 12 L/min; capillary voltage, capillary voltage, 4000 V and 3500 V in positive and negative modes, separately. The positive/negative polarity switching was used.

RPLC separation was performed by an ACQUITY UPLC HSS T3 (2.1×100 mm, 1.8 μm) column. The mobile phase A was water with 0.1% formic acid (v/v) and mobile phase B was acetonitrile with 0.1% formic acid (v/v) with a gradient elution: 0-2 min, 98% A; 2-10 min, 98%-65% A; 10-12 min, 65%-20% A; 12-14 min, 20%-2% A; 14-30 min, 2% A. And the equilibrium time was 15 min. HILIC separation using ACQUITY UPLC BEH Amide column (2.1 mm i.d×100 mm, 1.7 μm; Waters), the mobile phase A changed to water with 0.1% (v/v) formic acid and 10mM ammonium acetate, while mobile phase B was acetonitrile with 0.1% formic acid (v/v). The HILIC separation gradient was as follows: 0-4 min, 5% -12% A; 4-15min, 12%-50%; 15-25 min, 50% A. As the instability of amide column, the 15-min equilibrium time after a gradient run was necessary. The flow rate of mobile phase was 0.3 mL/min and the column temperature maintained at 40 ^{o}C. The samples were placed in 10 °C with a 5 μL-volume injection.

Samples preparation

In our protocol, the Urine samples could be analyzed by LC-TQ MS after a simple preparation: centrifuged to 12,000 g at 4 ^{o}C for 10 min and filtered by 0.22 μm Millipore filter. 100 ul of plasma samples were mixed with 4 vol of iced-cold acetonitrile to precipitate proteins, then centrifuged to 12,000 g for 10 min. Following the supernatant was transferred to a new 1.5-ml microcentrifuge tube and dried with

N_2, finally dissolved in 100 ul of water and analyzed by LC-TQ MS.

Tissues like brain, heart, liver, spleen, kidney, small intestine and lung share the same sample preparation procedures as followed. 100-130 mg tissue samples were weighed and placed into a 2-mL screw-cap plastic microvials containing about 1 mg glass beads (1.0 mm i.d.). Then tissues were completely homogenized at a 2-min run for 3 times (after one run we need to put the sample on ice for cooling) in 1.2 ml of 80% or ice-cold methanol by Mini-Beadbeater-16 (Biospec Products). After that, spun down at 12,000 g for 10 min and vortexed with 800 ul of iced-cold acetonitrile, centrifuged again to remove the protein precipitates. The supernatant was processed following the same procedure as plasma samples. Quality control (QC) samples by pooling aliquots of all samples were analyzed during the whole LC-TQ MS analysis.

Data Processing and Statistical Analysis

The MS raw data from the biological samples was processed by Qualitative Analysis, an in-house software of Agilent, which integrated the metabolite peaks and automatedly generated a peak area list of each metabolite. After normalized by tissue weight of each sample, the new data was uploaded to a free online clustering tool, MetaboAnalyst (http://www.metaboanalyst.ca/MetaboAnalyst/)[31] for partial least-squares discriminant analysis (PLS-DA) and heatmap hierarchical clustering.

Table 1. DMRM parameters of metabolites

Metabolites	Transition	CE	Polarity	Retention time (min)	Column	Quantification
Salicylic acid	139.0 /121.0	13	Positive	10.28	T3	AQ
L-Lactic acid	89.0 /45.2	9	Negative	1.51	T3	AQ
Pyruvate	87.0 /43.2	5	Negative	1.143	T3	AQ
Malic acid	133.0 /115.0	9	Negative	1.31	T3	AQ
Fumaric acid	115.0 /71.1	5	Negative	1.52	T3	AQ
Succinic acid	117.0 /73.1	9	Negative	2.31	T3	AQ
N-Acetylglutamic acid	188.05/128.0	9	Negative	1.88	T3	AQ
2-Ketobutyric acid	101.0 /57.2	5	Negative	1.82	T3	AQ
Creatine	132.1 /90.1	13	Positive	1.02	T3	AQ
L-Arginine	175.2 /70.2	29	Positive	0.96	T3	AQ
Indole-3-acetic acid	176.1 /130.1	17	Positive	10.23	T3	AQ
Acetylcoenzyme A	810.14/303.2	33	Positive	11.47	T3	RQ
Ethyl 4-hydroxybenzoate	167.2 /139.1	9	Positive	11.76	T3	AQ
Indole-3-propionic acid	190.2 /130.1	21	Positive	11.52	T3	AQ
Hippuric acid	180.2 /105.1	13	Positive	7.094	T3	AQ
Cinnamoylglycine	204.1 /160.1	9	Negative	9.51	T3	AQ
Chenodeoxycholic acid	391.3 /373.5	37	Negative	12.91	T3	AQ
Cholesterol	387.3 /105.0	25	Positive	12.74	T3	AQ
4-Hydroxybenzoic acid	137.02 /93.1	9	Negative	6.652	T3	AQ
2-Hydroxycinnamic acid	163.0 /119.1	9	Negative	9.693	T3	AQ
L-Valine	118.1 /72.2	9	Positive	1.54	T3	AQ
L-Leucine	132.2 /86.2	17	Positive	2.527	T3	AQ
Maleic acid	115.0 /71.1	9	Negative	1.62	T3	AQ
Eupatorin	345.3 /284.1	33	Positive	12.58	T3	AQ
Fmoc-cit-OH	398.4 /179.2	25	Positive	11.99	T3	AQ

Fmoc-cys(tbu)-OH	400.2 /179.0	29	Positive	13.41	T3	AQ
3-Isobutyl-1-methylxanthine	223.1 /167.0	21	Positive	9.3	T3	AQ
N-ethylmaleimide	126.1 /80.0	21	Positive	5.932	T3	AQ
Dimethyl 3,4-furandicarboxylate	185.2 /153.1	9	Positive	9.959	T3	AQ
Dimethyl(S)-malate	163.1 /103.1	5	Positive	5.56	T3	AQ
Lipoic acid	205.3 /171.0	5	Negative	12.25	T3	AQ
8-hydroxy-2-deoxyguanosine	284.1 /168.0	13	Positive	4.82	T3	AQ
5-Hydroxy-L-tryptophan	221.2 /204.1	9	Positive	4.34	T3	AQ
Itaconic acid	129.0 /85.1	5	Negative	4.65	T3	AQ
Indoxyl sulfate	212.0 /80.1	25	Negative	6.87	T3	AQ
Azelaic acid	187.1 /125.0	13	Negative	9.86	T3	AQ
Dexamethasone	393.2 /373.2	5	Positive	12.02	T3	AQ
Sebacic acid	201.1 /183.1	13	Negative	11.16	T3	AQ
N-Acetyl-L-cysteine	164.2 /122.1	5	Positive	3.38	T3	AQ
N-Acetyl-L-tryptophan	245.1 /203.1	13	Negative	9.04	T3	AQ
N-Acetyl-L-methionine	192.3 /144.1	9	Positive	6.197	T3	AQ
5-aminosalicylic acid	154.2 /136.0	13	Positive	1.54	T3	AQ
5-Methylcytidine	258.3 /126.2	17	Positive	1.605	T3	AQ
3-Phenylpropionylglycine	208.2 /105.1	21	Positive	9.19	T3	AQ
Uracil	113.1 /70.1	17	Positive	1.51	T3	AQ
Citraconic acid	129.1 /85.2	9	Negative	2.97	T3	AQ
Methylmalonic acid	117.0 /73.2	9	Negative	2.743	T3	AQ
Uridine	243.1 /199.9	9	Negative	2.009	T3	AQ
Deoxycytidine	228.2 /112.2	5	Positive	1.541	T3	AQ
Thymine	127.1 /110.1	13	Positive	2.92	T3	AQ
2-Deoxyguanosine	268.1 /152.1	9	Positive	3.75	T3	AQ
Adenine	136.1 /119.0	25	Positive	1.47	T3	AQ

2'-Deoxyinosine	251.1 /134.9	21	Negative	4.27	T3	AQ
Hypoxanthine	137.0 /55.2	33	Positive	1.57	T3	AQ
P-Coumaric acid	163.0 /119.2	13	Negative	8.48	T3	AQ
Rosmarinic acid	359.1 /161.0	13	Negative	9.87	T3	AQ
Tryptamine	161.1 /144.1	9	Positive	6.61	T3	AQ
Serotonin	177.2 /160.2	13	Positive	4.339	T3	AQ
L-Kynurenine	209.09/192.1	5	Positive	4.89	T3	AQ
L-Methionine	150.2 /104.2	9	Positive	1.482	T3	AQ
S-(5'-Adenosyl)-L-homocysteine	385.1/136.1	17	Positive	2.72	T3	AQ
Vanillic acid	167.0 /152.0	13	Negative	7.306	T3	AQ
3-Hydroxybenzoic acid	137.0 /93.0	9	Negative	6.9	T3	AQ
Biotin	245.3 /227.1	13	Positive	7.57	T3	AQ
Quercetin	303.1 /153.0	37	Positive	11.39	T3	AQ
Rotenone	395.4 /213.1	25	Positive	13.34	T3	AQ
Daidzein	255.1 /91.0	45	Positive	10.91	T3	AQ
Cyanocobalamin	678.5/147.1	49	Positive	6.85	T3	AQ
Lipoamide	206.4 /189.1	5	Positive	10.75	T3	AQ
6-Aminocaproic acid	132.1 /114.2	9	Positive	1.642	T3	AQ
Isonicotinic acid	124.1 /80.2	21	Positive	1.07	T3	AQ
Nicotinic acid	124.1 /80.2	21	Positive	1.51	T3	AQ
Protocatechualdehyde	139.0 /93.1	13	Positive	6.68	T3	AQ
Benzenesulfonic acid	157.0 /80.1	33	Negative	3.51	T3	AQ
Thiazolyl Blue	335.1 /77.0	25	Positive	11.997	T3	AQ
Caffeic acid	179.0 /135.1	17	Negative	7.47	T3	AQ
Pyridoxal	168.1 /150.0	13	Positive	1.7	T3	AQ
dUMP	307.03/194.7	13	Negative	1.6	T3	AQ
Cytidine monophosphate	324.2 /112.2	9	Positive	1.178	T3	AQ
2'-Deoxycytidine	308.1 /112.1	5	Positive	1.48	T3	AQ

5'-monophosphate						
Adenosine 5'-monophosphate	348.1 /136.0	25	Positive	1.51	T3	AQ
Inosine-5'-monophosphate	349.1 /136.9	9	Positive	1.57	T3	AQ
Guanosine 5'-monophosphate	364.1 /151.9	13	Positive	1.68	T3	AQ
Phenylpyruvate	163.0 /91.2	9	Negative	7.684	T3	AQ
Rifampin	823.4 /791.2	17	Positive	12.48	T3	AQ
1,2-diacyl-sn-glycero-3-phospho-l-serine	792.58/607.7	21	Positive	5.79	Amide	RQ
1,2-Dioctadecanoyl-sn-glycero-3-phospho-rac-(1-glycerol)	777.560/283.4	45	Negative	2.62	Amide	RQ
Dioleoyl-sn-glycero-3-phosphoethanolamine	744.560/603.6	25	Positive	3.35	Amide	RQ
L-α-phosphatidylcholine	758.6/184	37	Positive	3.81	Amide	RQ
Phosphoenolpyruvic acid	168.99/150.9	5	Positive	9.34	Amide	RQ
CoQ10	863.7/197	33	Positive	0.88	Amide	RQ
5-Methylcytosine	126.1 /109.0	21	Positive	4.99	Amide	RQ
D-Pantothenic acid	220.120/90.2	13	Positive	2.26	Amide	RQ
Shikimic acid	173/93	9	Negative	4.4	Amide	RQ
1,2-Dipalmitoyl-sn-glycero-3-phosphocholine	734.570/184.1	33	Positive	3.36	Amide	RQ
Sphingomyelin	703.7/184.1	25	Positive	4.51	Amide	RQ
FMOC-GLN(TMOB)-OH	549.6 /181.1	13	Positive	12.98	T3	RQ
Trimethoprim	291.2 /230.1	25	Positive	7.26	T3	RQ
Heptadecanoic acid	269.250/251	21	Negative	13.97	T3	RQ
Butylated hydroxytoluene	219.2/203.1	29	Negative	14.44	T3	RQ
L-Norleucine	132.1 /86.1	9	Positive	2.68	T3	RQ

N-Acetylglutamine	189.2 /130.2	9	Positive	1.6	T3	RQ
Gluconic acid	195.05/129.2	13	Negative	0.94	T3	RQ
Oxaloacetic acid	131/87.1	5	Negative	1.13	T3	RQ
Tryptophan	205.1/188.1	9	Positive	5.71	T3	RQ
Cytosine	112.1 /95.1	17	Positive	1.54	T3	RQ
2'-Deoxyuridine	229.2 /113.0	12	Positive	2.863	T3	RQ
Adenosine	268.3 /136.2	17	Positive	3.35	T3	RQ
2-Deoxyadenosine	252.1 /136.0	13	Positive	3.71	T3	RQ
2'-Deoxyguanosine 5'-monophosphate	348/152.1	13	Positive	1.6	T3	RQ
p-Hydroxyphenylpyruvate	181.05/135.1	9	Positive	8.01	T3	RQ
p-Hydroxyphenylpropionic acid	165.05/121.2	9	Negative	7.96	T3	RQ
4-Hydroxyphenylacetic acid	151.04/107.1	9	Negative	6.89	T3	RQ
3-Indoleacrylic acid	188.07/170	13	Positive	10.84	T3	RQ
2-Methylhippuric acid	194.08/119.1	9	Positive	7.7	T3	RQ
Syringic acid	199.06/140.1	13	Positive	7.37	T3	RQ
Pyrogallol	127.04/109.1	13	Positive	3.2	T3	RQ
Phenylpropionic acid	149.06/105.1	5	Negative	11.4	T3	RQ
2-hydroxy-2-phenylpropanoic acid	165.05/121	9	Negative	8.3	T3	RQ
Indolepyruvic acid	204.07/158.1	13	Positive	11.05	T3	RQ
3-Hydroxyphenylpropionic acid	165.05/121	9	Negative	8.44	T3	RQ
Trans-Cinnamic acid	147.04/103	9	Negative	11.53	T3	RQ
Taurocholic acid	514.3/107.1	49	Negative	11.66	T3	RQ
Glycocholic acid	464.3/74.1	45	Negative	12.08	T3	RQ
Lithocholic acid	375.3/375.3	0	Negative	14.15	T3	RQ
Taurochenodeoxycholic acid	498.3 /124.0	50	Negative	12.16	T3	RQ

Deoxycholic acid	391.3 /345.2	37	Negative	13.38	T3	RQ
Taurodeoxycholic acid	498.3/123.9	50	Negative	12.25	T3	RQ
Tauroursodeoxycholic acid	498.3/123.9	50	Negative	11.66	T3	RQ
L-Phenylalanine	166.2 /120.1	13	Positive	4.67	T3	RQ
Isoleucine	132.2 /86.2	9	Positive	2.699	T3	RQ
Dopamine	154.2 /137.1	9	Positive	1.71	T3	RQ
Thiamine	266.1 /122.1	21	Positive	0.93	T3	RQ
Oxidized glutathione	613.2/354.9	21	Positive	2.04	T3	RQ
Glutathione	308.1 /178.9	9	Positive	1.59	T3	RQ
Beta-Sitosterol	415.4/119.2	29	Positive	13.08	T3	RQ
L-Carnitine	162.1 /103	17	Positive	1.1	T3	RQ
Normetanephrine	184.1/166	5	Positive	1.55	T3	RQ
Palmitoyl-l-carnitine	400.3/85.1	33	Positive	12.871	T3	RQ
Sphinganine	302.3/284.2	13	Positive	12.9	T3	RQ
Flavin adenine dinucleotide	784.150/436.9	29	Negative	6.23	T3	RQ
Uridine 5'-monophosphate	325.1 /97.0	9	Positive	1.13	T3	RQ
Acetylcholine	147.1 /87.1	13	Positive	1.19	T3	RQ
Dephospho-CoA	688.2/261.1	25	Positive	5.99	T3	RQ
Aminobutyric acid	104.1 /87.2	9	Positive	0.933	T3	RQ
2-Aminoimidazole	84.1 /42.2	28	Positive	1.033	T3	RQ
Allantoin	157.030/114.1	9	Negative	0.99	T3	RQ
1-Naphthylacetamide	186.230/141.1	21	Positive	10.85	T3	RQ
2,4-Diaminobutyric acid	119.1/101.2	5	Positive	0.828	T3	RQ
3-Guanidinopropionic acid	132.14/72.2	17	Positive	1.24	T3	RQ
D-Glucose 6-phosphate	259.020/97.1	17	Negative	0.89	T3	RQ
2-Ketoglutaric acid	145/101.1	5	Negative	1.483	T3	RQ
Cis-Aconitic acid	173.0 /111.1	5	Negative	1.9	T3	RQ
L-Aspartic acid	134.1/74.2	13	Positive	1.05	T3	RQ
L-Glutamic acid	148.1 /130.1	5	Positive	2.06	T3	RQ

L-Citrulline	176.2 /70.2	25	Positive	0.94	T3	RQ
N-Acetylornithine	175.2 /115.2	9	Positive	0.97	T3	RQ
L-Proline	116.1 /70.2	17	Positive	0.999	T3	RQ
Creatinine	114.07/86.1	9	Positive	1	T3	RQ
Putrescine	89.1 /72.2	5	Positive	0.76	T3	RQ
Sarcosine	90.1 /44.0	13	Positive	0.9	T3	RQ
Trans-4-Hydroxy-L-proline	132.1 /86.2	13	Positive	0.907	T3	RQ
Glyoxylic acid	72.99/45.2	5	Negative	0.9	T3	RQ
Betaine	118.2 /59.2	17	Positive	0.95	T3	RQ
Betaine aldehyde	102.2/58.3(59.2)+	20	Positive	0.9	T3	RQ
5-Aminolevulinic acid	132.1 /114.1	5	Positive	0.96	T3	RQ
L-Cystathionine	223.3 /88.1	29	Positive	0.88	T3	RQ
L-Threonine	120.1 /74.2	9	Positive	0.919	T3	RQ
L-Homoserine	120.1 /74.1	9	Positive	0.91	T3	RQ
Cytidine	244.2 /112.1	9	Positive	1.25	T3	RQ
Glycine	76.040/30.2	17	Positive	0.892	T3	RQ
Trimethylamine N-oxide	76.2 /58.2	21	Positive	0.915	T3	RQ
Glycochenodeoxycholic acid	448.3/386.1	37	Negative	12.64	T3	RQ
Cholic acid	407.3 /343.1	37	Negative	12.64	T3	RQ
Taurine	126.2/108.1	9	Positive	0.89	T3	RQ
Choline	105.1/61.2	17	Positive	0.874	T3	RQ
L-Carnosine	227.2 /210.2	9	Positive	0.93	T3	RQ
L-Cysteic acid	170.170/124.1	13	Positive	0.885	T3	RQ
L-Homocysteine	136.05/90.1	9	Positive	1.11	T3	RQ
Alanine	90.1 /44.3	17	Positive	0.9	T3	RQ
Glucosamine	180.1/162.1	5	Positive	0.85	T3	RQ
Glycolic acid	75.0 /47.2	9	Negative	1.08	T3	RQ
L-Ascorbic acid	177.0/95.1	9	Positive	1.435	T3	RQ

L-Lysine	147.2 /130.2	9	Positive	0.8	T3	RQ
Cysteamine	78.04/61.0	21	Positive	0.778	T3	RQ
Hypotaurine	110.03/92.1	5	Positive	0.78	T3	RQ
Agmatine	131.1 /72.2	17	Positive	0.88	T3	RQ
Inosine	269.09/137.1	29	Positive	3.25	T3	RQ
Histamine	112.1 /95.1	13	Positive	0.996	T3	RQ
L-Histidine	156.2 /110.1	13	Positive	0.84	T3	RQ
Ursodeoxycholic acid	391.280/373.3	33	Negative	12.69	T3	RQ
Beta-Glycerophosphate	171/79	45	Negative	0.93	T3	RQ
D-Tartaric acid	149.01/86.9	13	Negative	1.02	T3	RQ
Roxithromycin	837.5 /158.0	33	Positive	11.8	T3	RQ
Hexanoylcarnitine	260.1 /85.1	21	Positive	8.69	T3	RQ
Glucose	179.05/89	5	Negative	0.94	T3	RQ
2-Phospho-D-glyceric acid	184.98/78.9	29	Negative	1.2	T3	RQ
DHAP	168.9/96.9	9	Negative	0.95	T3	RQ
D-Glyceraldehyde 3-phosphate	168.99/97.2	9	Negative	0.99	T3	RQ
Citrate	191.1 /111.0	9	Negative	1.655	T3	RQ
Succinyl coenzyme A	868.14/361.2	41	Positive	5.39	T3	RQ
Guanidineacetic acid	118.1 /72.1	9	Positive	0.95	T3	RQ
Spermidine	146.2 /72.2	17	Positive	0.71	T3	RQ
Malonic acid	103/59.2	9	Negative	1.36	T3	RQ
Glycodeoxycholic acid	450/414.2	13	Positive	12.74	T3	RQ
L-Histidinol	142.1 /124.1	9	Positive	1	T3	RQ
Pyridoxal 5'-phosphate	248.2 /150.0	17	Positive	1.63	T3	RQ
D-Fructose	179.1 /89.1	5	Negative	1.13	T3	RQ
Acetylcarnitine	204.2 /85.1	17	Positive	1.54	T3	RQ
Adenylosuccinic acid	464.1 /251.9	21	Positive	4.3	T3	RQ
NADH	664.1/407.8	33	Negative	4.12	T3	RQ

Oxalic acid	89/45.2	5	negative	0.91	T3	RQ

AQ: Absolute Quantification
RQ: Relative Quantification

Table 2. absolute quantification of 84 key metabolites

Metabolites	Linear range (ng/mL)	Calibration curve	R^2
Salicylic acid	2.5-1000	Y=2624.2X-4610.3	0.9998
Vanillic acid	2.5-1000	Y=2163.5X-10209.8	0.9998
Nicotinic acid	2.5-500	Y=47649.3X+192890	0.9997
Indole-3-propionic acid	2.5-500	Y=22900.9X+46815.8	0.9994
dUMP	12.5-1000	Y=955.7X-5855	0.9994
N-Acetylglutamic acid	2.5-1000	Y=1594.3X-4996.2	0.9991
Cinnamoylglycine	2.5-1000	Y=1725.5X+8798.5	0.9989
Benzenesulfonic acid	2.5-1000	Y=5340.4X+38032.9	0.9989
4-Hydroxybenzoic acid	2.5-1000	Y=1543X+9454.9	0.9988
3-Isobutyl-1-methylxanthine	2.5-250	Y=114641.8X+40652.2	0.9986
5-aminosalicylic acid	2.5-250	Y=73980.1X+157966.2	0.9986
Cyanocobalamin	2.5-1000	Y=8441.8X-95729.5	0.9985
Fumaric acid	2.5-1000	Y=596.2X+6360	0.9985
L-Kynurenine	2.5-1000	Y=18094.3X+97616.3	0.9984
Creatine	1.56-100	Y=54009.2X+144085.6	0.9984
P-Coumaric acid	2.5-1000	Y=2371.3X+28667.1	0.9982
N-Acetyl-L-tryptophan	2.5-1000	Y=2626.1X+18657.1	0.9981
Biotin	2.5-1000	Y=15839.5X+4783.6	0.9979
2-Hydroxycinnamic acid	2.5-1000	Y=1995.3X+23969.8	0.9975
Citraconic acid	10-1250	Y=1281.4X+19737.9	0.9975
Indoxyl sulfate	2.5-1000	Y=3994.7X+48714.7	0.9971
Lipoic acid	0.5-50	Y=9109.7X-11507.9	0.9967
Hippuric acid	5-500	Y=42888.8X+158271.3	0.9966
Chenodeoxycholic acid	62.5-1000	Y=4.2X-354.4	0.9965
2-Deoxyguanosine	2.5-1000	Y=26263X+651590.1	0.9963
Caffeic acid	2.5-500	Y=6884.7X+65740.9	0.9963
N-Acetyl-L-cysteine	2.5-500	Y=9459X-105383.1	0.9962
Cholesterol	0.5-50	Y=11407.8X+10664.3	0.9962
Rotenone	0.5-50	Y=31916X-13227.1	0.9959
Thymine	50-1000	Y=4804.1X+147480.3	0.9956
2'-Deoxyinosine	10-1250	Y=4086.1X+133624.3	0.9954
Cytidine monophosphate	2.5-1000	Y=11123.9X-158399.7	0.9951
Fmoc-cys(tbu)-OH	2.5-1000	Y=3137.4X-63556.6	0.995
Maleic acid	2.5-1000	Y=3884.6X+87802.4	0.9948

2'-Deoxycytidine 5'-monophosphate	2.5-1000	Y=8828.8X-233907.4	0.9947
Isonicotinic acid	2.5-500	Y=119179.9X+1848509.5	0.9947
Phenylpyruvate	10-1250	Y=568.5X-6634.7	0.9945
Fmoc-cit-OH	0.5-50	Y=325329.9X-143312	0.9944
3-Phenylpropionylglycine	5-500	Y=52539.4X+256602.1	0.9942
Rosmarinic acid	5-2000	Y=5588.7X+97664.4	0.9934
Methylmalonic acid	10-1250	Y=381.7X+17855.8	0.9931
N-Acetyl-L-methionine	2.5-1000	Y=7069.9X+128333.7	0.993
Dexamethasone	2.5-1000	Y=6594.6X+77157.6	0.9929
Protocatechualdehyde	2.5-1000	Y=6305.3X+142590.6	0.9929
3-Hydroxybenzoic acid	2.5-1000	Y=3881.4X+92292.5	0.9927
Serotonin	2.5-1000	Y=13867.7X+134201.9	0.9924
Adenine	2.5-250	Y=67087.2X+790897.1	0.9923
Itaconic acid	2.5-1000	Y=1532.1X+39902.8	0.9919
Dimethyl 3,4-furandicarboxylate	5-100	Y=327660.7X+1391516.5	0.9918
Ethyl 4-hydroxybenzoate	5-500	Y=35687.8X+372792.2	0.9918
Lipoamide	2.5-500	Y=12797X+169419.1	0.9913
5-Hydroxy-L-tryptophan	1.56-100	Y=24068.5X-92904.3	0.9912
Uridine	10-1250	Y=387.9X+18567.6	0.9912
8-hydroxy-2-deoxyguanosine	1.56-100	Y=14740X-56280.7	0.9907
Guanosine 5'-monophosphate	5-1000	Y=3726.7X-77860.9	0.9904
Adenosine 5'-monophosphate	2.5-1000	Y=10429.6X-322495.8	0.9903
Dimethyl(S)-malate	50-1000	Y=9642.1X+869793.2	0.99
L-Arginine	62.5-1000	Y=23681.3X-465300.4	0.99
Thiazolyl Blue	5-100	Y=117733.8X+471535.6	0.9888
5-Methylcytidine	2.5-500	Y=33341.3X+717161.8	0.9886
Uracil	5-500	Y=19981.8X+318592.6	0.9885
Rifampin	12.5-500	Y=87.8X-4012.2	0.9884
L-Methionine	2.5-1000	Y=3650.2X+114769.8	0.9883
Azelaic acid	2.5-1000	Y=3641.3X+136598.4	0.9882
Inosine-5'-monophosphate	2.5-500	Y=367.7X-6993.7	0.9878
Hypoxanthine	62.5-1000	Y=8037.4X+1575299.5	0.9871
Deoxycytidine	62.5-1000	Y=5231.1X+1573951.7	0.9866
N-ethylmaleimide	6250-80000	Y=162.6X+6115828.6	0.9865
6-Aminocaproic acid	2.5-500	Y=22150.4X+639770.1	0.9861
L-Valine	2.5-500	Y=32151.5X+521586.5	0.9845
Sebacic acid	5-500	Y=10385.5X+239366.4	0.9845
S-(5'-Adenosyl)-L-homocysteine	2.5-1000	Y=22127.8X+986168.4	0.9842
2-Ketobutyric acid	10-1250	Y=433.1X+29981.2	0.984
Eupatorin	0.5-50	Y=73582.7X+133465	0.9831
L-Leucine	2.5-1000	Y=41002.8X+812054.5	0.9829
Indole-3-acetic acid	1.56-100	Y=9099.2X-46024.3	0.9826
Pyridoxal	50-1000	Y=32722.9X+5582005	0.9824
Tryptamine	2.5-250	Y=72155.2X+1575771.6	0.9822

Daidzein	2.5-250	Y=21948.8X+367723.7	0.9819
Quercetin	12.5-1000	Y=810.5X-27194.9	0.9809
Malic acid	12.5-1000	Y=1693.7X-75073.9	0.9808
Succinic acid	6250-80000	y = 157.3X + 4139425.2	0.9795
Pyruvate	1250-80000	Y=18.9X+115530.6	0.9799
L-Lactic acid	1250-80000	Y=2.7X+119781.5	0.9600

Table 3. Concentration of 27 key metabolites in plasma, urine and different tissues of rats (n=6)

Metabolites	Concentration (ng/mL)								
	Brain	Heart	Lung	Spleen	Kidney	Liver	Small intestine	Plasma	Urine
Rotenone	0.52±0.03	0.46±0.02	0.45±0.01	0.45±0.01	0.51±0.04	0.48±0.02	0.43±0.01	0.45±0.01	0.44±0.01
Lipoic acid	1.88±0.04	2.52±0.14	1.95±0.04	2.1±0.05	1.87±0.07	2.17±0.15	1.86±0.11	1.91±0.05	27.27±5.83
Eupatorin	3.2±0.3	541.1±538.39	626.87±620.42	3.9±0.42	9194.59±3281.69	2259.3±1010.62	3022.36±606.24	1317.92±589.75	60.6±36.14
5-Hydroxy-L-tryptophan	4.13±0.02	4.31±0.08	4.04±0.03	4.12±0.04	4.92±0.08	4.75±0.1	4.78±0.09	3.87±0.01	4.04±0.04
8-hydroxy-2-deoxyguanosine	24.84±19.75	41.84±34.12	78.11±72.75	236.3±73.71	7.28±1.28	356.99±45.33	7±1.08	5.8±0.32	47.27±9.76
dUMP	13.2±2.02	16.53±1.66	15.68±1.69	13.85±1.5	16.12±1.47	21.35±1.93	22.34±3.76	19.32±0.51	10696.48±2928.27
N-Acetylglutamic acid	13.87±2.63	324.95±82.88	7.68±0.7	7.79±0.68	486.43±102.85	288.67±81.64	16±4.3	11.3±0.61	617.35±486.15
Inosine-5'-monophosphate	21.6±0.58	21.06±0.19	21.56±0.46	21.36±0.37	20.41±0.13	20.51±0.14	21.43±0.43	26.26±0.34	24.04±2.38
Indole-3-acetic acid	31.7±3.38	139.92±117.65	52.29±2.34	1021.61±943.96	22.74±1.55	23.14±1.53	30.51±4.24	34.07±8.65	78.62±46.97
Adenosine 5'-monophosphate	32.18±0.15	44.08±2	52.7±2.03	37.65±0.81	47.9±2.51	38.25±1.45	33.64±0.64	123.55±8.1	582.92±143.7
Malic acid	45.04±0.11	46.91±0.13	45.92±0.11	46.3±0.19	45.76±0.19	45.99±0.19	147.36±101.65	44.58±0.26	55.01±3.33
2'-Deoxycytidine 5'-monophosphate	42.75±2.53	32.61±0.41	38.98±0.72	33.92±0.83	27.43±0.09	29.03±0.43	28.8±0.93	78.11±19.71	26.52±0.02
Cytidine monophosphate	37.33±3.75	28.95±4.77	25.92±1.12	25.02±0.92	42.22±2.04	43.14±2.35	20.56±0.42	62.53±6.02	181.63±54.53

4-Hydroxybenzoic acid	76±5.7	78.81±13.05	174.69±19.68	41.49±2.73	84.56±13.25	62.43±7.49	144.86±19.22	32.38±13.73	857.02±224.95
N-Acetyl-L-cysteine	37.96±7.55	644.03±115.07	17.6±1.18	29.68±6.99	13.05±0.2	74.97±26.86	37.81±4.98	12.13±0.32	11.3±0.09
Vanillic acid	96.28±4.39	804.15±25.77	154.4±6.7	239.31±6.72	116.52±3.58	102.68±5.27	212.9±18.98	198.56±11.96	34.89±10.87
Guanosine 5'-monophosphate	93.39±2.78	596.36±18.85	142.85±5.72	196.94±2.54	108.45±2.73	93.95±2.57	185.91±14.92	173.04±6.84	42.48±7.79
Cinnamoylglycine	112.2±10.15	225.28±17.49	238.61±26.38	234.87±7.96	74.65±7.83	36±1.88	173.11±29.4	304.92±28.21	29.02±23.39
Quercetin	128.4±8.51	44.96±1.06	44.44±1.76	173.67±6.85	45.33±1.05	36.88±1.37	325.05±72.54	104.27±5.69	50.08±6.27
L-Arginine	175.28±13	504.46±19.82	359.11±12.68	387.61±28.05	890.46±26.09	551.9±27.81	753.88±26.58	52.49±5.05	27.62±3.35
L-Kynurenine	179.04±2.83	243.77±8.9	185.54±8.98	156.34±3.77	156.21±6.63	268.27±8.76	1266±711.29	372.66±204.8	1353.53±432.66
Dexamethasone	245.88±13.56	187.32±13.2	397.53±20.03	636.78±44.93	171.75±10.15	148.27±14.69	219.14±35.62	52.71±26.64	3.87±14.3
Cholesterol	246.48±18.86	141.6±18.48	123.73±13.58	206.33±16.52	329.03±40.04	264.85±28.64	177.72±45.05	9.5±1.67	15.73±3.7
Ethyl 4-hydroxybenzoate	259.24±23.79	555.57±28.89	551.43±25.09	1048.87±30.15	1469.62±37.4	649.06±25.24	580.59±127.45	1078.29±54.71	57.45±14.2
Chenodeoxycholic acid	378.82±78.25	606.96±196.07	931.17±358.64	260.93±50.92	489.91±90.74	12102.13±1412.05	14128.96±5535.57	277.26±51.01	90.44±2.21
Creatine	722.5±15.54	687.38±12.01	455.58±28.5	679.24±21.13	442.86±89.19	733.66±20.62	140.5±62.14	1.28±0.86	0.13±1.52
Cyanocobalamin	768.09±56.44	1396.83±221.68	601.39±137.56	465.06±20.08	495.35±34.73	663.14±122.32	606.12±27.64	51.71±5.28	33.62±10.46

ACKNOWLEDGEMENTS

This work was supported by National Natural Science Foundation of China grants (No. 81274175 and c010201), National Key R&D Program of China (No. 2017YFC1308600), and the Startup Funding for Specialized Professorship provided by Shanghai Jiao Tong University (No. WF220441502).

References

1 Jang, C., Chen, L. & Rabinowitz, J. D. Metabolomics and Isotope Tracing. *Cell* **173**, 822-837, doi:10.1016/j.cell.2018.03.055 (2018).

2 Boulange, C. L., Neves, A. L., Chilloux, J., Nicholson, J. K. & Dumas, M. E. Impact of the gut microbiota on inflammation, obesity, and metabolic disease. *Genome Med* **8**, 42, doi:10.1186/s13073-016-0303-2 (2016).

3 Geiger, R. *et al.* L-Arginine Modulates T Cell Metabolism and Enhances Survival and Anti-tumor Activity. *Cell* **167**, 829-842.e813, doi:10.1016/j.cell.2016.09.031 (2016).

4 Oliver, S. G., Winson, M. K., Kell, D. B. & Baganz, F. Systematic functional analysis of the yeast genome. *Trends Biotechnol* **16**, 373-378, doi:Doi 10.1016/S0167-7799(98)01214-1 (1998).

5 Nicholson, J. K., Lindon, J. C. & Holmes, E. 'Metabonomics': understanding the metabolic responses of living systems to pathophysiological stimuli via multivariate statistical analysis of biological NMR spectroscopic data. *Xenobiotica* **29**, 1181-1189, doi:Doi 10.1080/004982599238047 (1999).

6 Winder, C. L., Dunn, W. B. & Goodacre, R. TARDIS-based microbial metabolomics: time and relative differences in systems. *Trends Microbiol* **19**, 315-322, doi:10.1016/j.tim.2011.05.004 (2011).

7 Want, E. J. *et al.* Global metabolic profiling of animal and human tissues via UPLC-MS. *Nature protocols* **8**, 17-32, doi:10.1038/nprot.2012.135 (2013).

8 Want, E. J. *et al.* Global metabolic profiling procedures for urine using UPLC-MS. *Nature protocols* **5**, 1005-1018, doi:10.1038/nprot.2010.50 (2010).

9 Kuo, M. T., Savaraj, N. & Feun, L. G. Targeted cellular metabolism for cancer chemotherapy with recombinant arginine-degrading enzymes. *Oncotarget* **1**, 246-251, doi:10.18632/oncotarget.135 (2010).

10 Luo, X. *et al.* Mass spectrometry and associated technologies delineate the advantageously biomedical capacity of siderophores in different pathogenic contexts. *Mass Spectrometry Reviews* **0**, doi:doi:10.1002/mas.21577.

11 Cai, Y., Weng, K., Guo, Y., Peng, J. & Zhu, Z.-J. An integrated targeted metabolomic platform for high-throughput metabolite profiling and automated data processing. *Metabolomics* **11**, 1575-1586,

doi:10.1007/s11306-015-0809-4 (2015).

12 Wei, R., Li, G. D. & Seymour, A. B. High-Throughput and Multiplexed LC/MS/MRM Method for Targeted Metabolomics. *Analytical chemistry* **82**, 5527-5533, doi:10.1021/ac100331b (2010).

13 Su, Q., Guan, T., He, Y. & Lv, H. Siderophore Biosynthesis Governs the Virulence of Uropathogenic Escherichia coli by Coordinately Modulating the Differential Metabolism. *Journal of proteome research* **15**, 1323-1332, doi:10.1021/acs.jproteome.6b00061 (2016).

14 Lv, H., Hung, C. S., Chaturvedi, K. S., Hooton, T. M. & Henderson, J. P. Development of an integrated metabolomic profiling approach for infectious diseases research. *Analyst* **136**, 4752-4763, doi:10.1039/c1an15590c (2011).

15 Lv, H. *et al.* Ingenuity pathways analysis of urine metabonomics phenotypes toxicity of gentamicin in multiple organs. *Mol Biosyst* **6**, 2056-2067, doi:10.1039/c0mb00064g (2010).

16 Domingo-Almenara, X. *et al.* XCMS-MRM and METLIN-MRM: a cloud library and public resource for targeted analysis of small molecules. *Nat Methods* **15**, 681-684, doi:10.1038/s41592-018-0110-3 (2018).

17 Rao, Z. *et al.* Development of a dynamic multiple reaction monitoring method for determination of digoxin and six active components of Ginkgo biloba leaf extract in rat plasma. *Journal of chromatography. B, Analytical technologies in the biomedical and life sciences* **959**, 27-35, doi:10.1016/j.jchromb.2014.03.028 (2014).

18 Lv, H., Palacios, G., Hartil, K. & Kurland, I. J. Advantages of tandem LC-MS for the rapid assessment of tissue-specific metabolic complexity using a pentafluorophenylpropyl stationary phase. *Journal of proteome research* **10**, 2104-2112, doi:10.1021/pr1011119 (2011).

19 Feng, Y. *et al.* Lactate dehydrogenase A: A key player in carcinogenesis and potential target in cancer therapy. *Cancer Med* **7**, 6124-6136, doi:10.1002/cam4.1820 (2018).

20 Lou, Y. B., Fan, F. X., Mu, Y. C. & Dong, X. The Implication of Diabetes Metabolomics in the Early Diagnosis and Pathogenesis of Pancreatic Cancer. *J Biol Reg Homeos Ag* **32**, 75-82 (2018).