Supporing Information

Metabolomics-based evidence of the hypoglycemic effect and alleviation of diabetic complications by *Ficus racemosa* fruit in diabetic mice

Yueqiu Liu[a, c], Wen Zheng[a], Lu Zhang[a], Liqiang Hu[a], Xin Liu[a], Jingqiu Cheng[a], Guoliang Li[b*], Meng Gong[a*]

[a] Laboratory of Clinical Proteomics and Metabolomics, Institutes for Systems Genetics, Frontiers Science Center for Disease-Related Molecular Network, National Clinical Research Center for Geriatrics, West China Hospital, Sichuan University, Chengdu, Sichuan, China

[b] School of Food and Biological Engineering, Shaanxi University of Science and Technology, Xi'an, China

[c] College of Materials and Chemistry & Chemical Engineering, Chengdu University of Technology, Chengdu, China

[*] *Corresponding author:*

Meng Gong

E-mail: gongmeng@scu.edu.cn;

Guoliang Li

Email: 61254368@163.com

Chemicals and reagents

The fruits of *Ficus racemosa* were purchased from Southwest Treasure Herbs (Chengdu, China), where the voucher specimen was deposited. LC-MS-grade formic acid, acetonitrile, methanol and sterilized phosphate buffered saline (PBS) were purchased from Fisher Scientific (Hampton, NH, USA). LC-MS grade acetic acid, ammonium formate, ammonium acetate and analytical grade acarbose, hematoxylin and eosin (H&E), streptozotocin (STZ), sodium citrate and citric acid were ordered from Sigma-Aldrich (St. Louis, MO, USA).

Plasma sample preparation

For sample preparation, 50 µl of plasma was mixed with 250 µl of spiked methanol by vortexing for 30 min and centrifuging at 17,000 g for 20 min at 4 °C. The supernatant was concentrated under vacuum to dryness and stored at -80 °C for metabolite analysis. Prior to the metabolite analysis, the extracts were reconstituted in 200 µl of mobile phase mixture (solvent A-solvent B, 20:80, v/v).

Tissue sample preparation

Ten milligrams of tissue sample, 200 µL of precooled MeOH/H2O (4:1) solution, and 6 steel balls were mixed and homogenized 4 times at 4 °C (30 s per time). Then, 800 µL of spiked methanol solution was added and swirled for 3 min at 1500 rpm (4 °C). The supernatant was collected after centrifugation. The samples were subsequently placed in the refrigerator for 30 min, sonicated in an ice water bath for 10 min, swirled for 3 min and centrifuged at 13300 rpm at 4 °C for 15 min. An 800 µL volume of the supernatant was transferred into an EP tube. Another 500 µL of MeOH/H2O (4:1) solution was added to the residue, swirled for 3 min and centrifuged at 13300 rpm at 4 °C for 15 min. Four hundred microliters of the supernatant was collected and combined with the former supernatant in an EP tube. Lastly, the extracts were concentrated to dryness under a vacuum and stored at -80 °C for metabolite analysis. The extracts were redissolved in 500 µL of HILIC solution (60% acetonitrile with 5 mM ammonium formate), swirled for 3 min, sonicated in an

ice water bath for 10 min, and centrifuged at 13300 rpm at 4 °C for 10 min. Then, 150 μL of the supernatant was obtained for metabolite analysis. A mixture containing 30 μL of each sample was prepared as the quality control (QC).

UPLC-MS/MS conditions for plasma metabolite profiling

Plasma metabolic profiling analysis was performed on an Ultimate 3000 rapid separation liquid chromatograph coupled with Q Exactive Plus Q-Orbitrap HRMS (Thermo Fisher Scientific, Waltham, MA, USA) by using a BEH Amide column (2.1 × 100 mm, 1.7 m) (Waters, Milford, MA, USA). For the chromatographic separation, the mobile phase was composed of phase A with 0.2% acetic acid and 10 mM ammonium formate in water and phase B with 90% acetonitrile in water (including 0.15% formic acid and 10 mM ammonium formate). The gradient elution was as follows: 0~2 min, 100% B; 2~9 min, 100~85% B; 9~14 min, 85~50% B; 14~17 min, 50% B for column cleaning; and 17~25 min, 100% B for column re-equilibration. The flow rate was 0.3 mL/min, the column temperature was maintained at 40 °C, and the sample injection volume was 2 μL. The ion source parameters were as follows: sheath gas flow at 35 arb, auxiliary gas flow at 10 arb with a heater temperature at 350 °C, s-lens RF level at 60%, capillary temperature at 320 °C, ion spray voltage at + 3200 V for positive mode and - 3200 V for negative mode.

UPLC-MS/MS conditions for tissue metabolite profiling

Tissue metabolic profiling analysis was performed on a Shimadzu LC-30A liquid chromatograph (Shimadzu, Kyoto, Japan) with a Waters Acquity UPLC BEH Amide column (2.1 mm×100 mm, 1.7 μm, Waters, Milford, USA) coupled with an AB Sciex 5500 triple quadrupole mass spectrometer (AB SCIEX, Framingham, USA). A total of 228 metabolites (99 in positive mode and 128 in negative mode) chosen for the targeted analysis represented the major metabolic pathways in the diabetic alleviation effect when using multiple reaction monitoring mode. For chromatographic separation, the mobile phase was composed of phase A with 10% acetonitrile in water (containing 0.2% acetic acid and 10 mM ammonium acetate) and phase B with 90% acetonitrile

in water (including 0.2% acetic acid and 10 mM ammonium acetate). The gradient elution was as follows: 0.0~0.5 min: 0~10% A; 0.5~1.5 min: 10% A; 1.5~5.0 min: 10~55% A; 5.0~10.0 min: 55% A; 10.0~12.0 min: 55~10% A; and 12.0~25.0 min: 10% A. The flow rate was 0.3 mL/min, the column temperature was maintained at 40 °C, and the sample injection volumes were 5 μL and 15 μL for positive and negative modes, respectively. The ion source parameters were as follows: sheath gas flow at 50 psi, aux gas flow at 40 psi, curtain gas flow at 35 psi, interface capillary temperature at 650 °C, ion spray voltage at + 4000 V for positive mode and - 4000 V for negative mode.

Statistical analysis

The raw metabolic profile data of tissues were imported into MultiQuant (v2.0.3) for integration. The metabolites of plasma were identified with available reference standards in our lab and the web-based resources, e.g., the Human Metabolome Database (http://www.hmdb.ca/) and METLIN (http:// metlin.scripps.edu/index.php) data source. The pretreated data, including the sample information, metabolite identity, and peak intensities, were then processed in R software (v3.5.1, Vienna, Austria, https://www.r-project.org/), in which compounds with missing values > 50% and coefficients of variation (CVs) > 20% for metabolites in the QC samples were filtered and treated with the k-nearest neighbor algorithm for missing value imputation. Subsequently, a principal component analysis (PCA) and partial least squares discrimination analysis (PLS-DA) were performed after median normalization to discriminate among samples with different treatments. Mfuzz soft clustering (https://bioconductor.org/packages/release/bioc/html/Mfuzz.html) was used to uncover the major clusters of the significantly changed molecules based on fuzzy c-means. The optimal cluster number was determined based on the mininum centroid distance. A metabolic pathway analysis was performed by using MetaboAnalyst 5.0 (Quebec, Canada, https://www.metaboanalyst.ca/home.xhtml) according to the pathway topology analysis together with pathway enrichment analysis through the Kyoto Encyclopedia of Genes and Genomes (www.genome.jp/kegg/) database to

identify the representative metabolic pathways. Kruskal-Wallis test was performed to investigate alterations among multiple groups and Wilcox test was performed for comparing the data between two groups.

Figure Legends

Fig. S1. Effects of *Ficus racemosa* fruit on PPG tested on day 8 (a), day 16 (d), day 24 (c), day 32 (d), day 40 (e).

Fig. S2. Effects of *Ficus racemosa* fruit on kidney pathological changes by using H&E staining method; Comparison between the model group (E1), high-dose group (E2), low-dose group (E3), postive group (E4) and control group (E5).

Fig. S3. Typical LC-MS/MS TIC spectra of tissue metabolic profiles. Pos: LC-MS/MS detection with positive mode, neg: LC-MS/MS detection with negative mode.

Fig. S4. The percentage of the variables with a relative standard deviation (RSD) below 20% in both ESI positive and negative ion mode in (A) liver, (B) kidney and (C) muscle.

Fig. S5. PCA and PLS-DA score plots of PC1 vs. PC2 based on the LC-MS/MS data of liver, kidney and muscle.

Fig. S6. Identification of specific clusters of metabolites in kidney (A) and muscle (B) using Mfuzz analysis. Profiles are represented by metabolite expression changes and the high-dose group, model group, control group.

Fig. S7. (A) Fold change of various representative metabolites in model group and high-dose group compared with control group in the kidney, (B) Fold change of various representative metabolites in model group and high-dose group compared with control group in the muscle, (C) The pathway analysis of metabolites in model group, high-dose group and control group in kidney. The node color is based on its p-value, and the node radius is determined based on their pathway impact values, (i) aminoacyl-tRNA biosynthesis; (d) arginine biosynthesis; (f) histidine metabolism, (D) The pathway analysis of metabolites in model group, high-dose group and control group in muscle, (l) glycine, serine and threonine metabolism; (a) purine metabolism; (g) TCA cycle. *$P < 0.05$, **$P < 0.01$, significantly different to model group.

Fig. S8. Fold change of various representative metabolites in high-dose, low-dose, model and postive group compared with control group in the plasma. (A) acetylcholine; (B) asparagine; (C) choline; (D) creatine; (E) glutamic acid; (F) histidine; (G) methyhistamine; (H) serine; (I) threonine.

Fig. S9. Typical LC-MS/MS TIC spectra of plasma metabolic profiles. Positive mode: LC-MS/MS detection with positive mode, negative mode: LC-MS/MS detection with negative mode.

Fig. S10. Typical DESI-MSI TIC spectra of liver sections including model, positive, high-dose, low-dose and control group. Pos: detection with positive mode, neg: detection with negative mode.

Fig. S11. Typical DESI-MSI TIC spectra of kidney sections including model, positive, high-dose, low-dose and control group. Pos: detection with positive mode, neg: detection with negative mode.

Fig. S12. Typical DESI-MSI TIC spectra of muscle sections including model, positive, high-dose, low-dose and control group. Pos: detection with positive mode, neg: detection with negative mode.

Tables

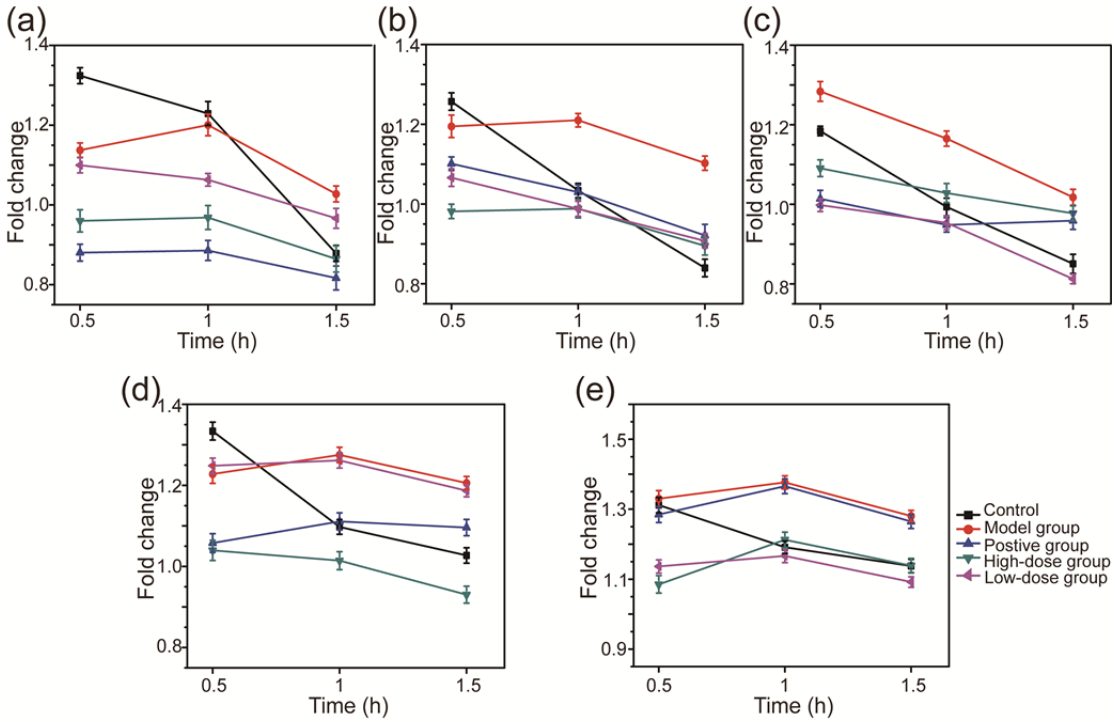

Fig. S1. Effects of *Ficus racemosa* fruit on PPG tested on day 8 (a), day 16 (b), day 24 (c), day 32 (d), day 40 (e).

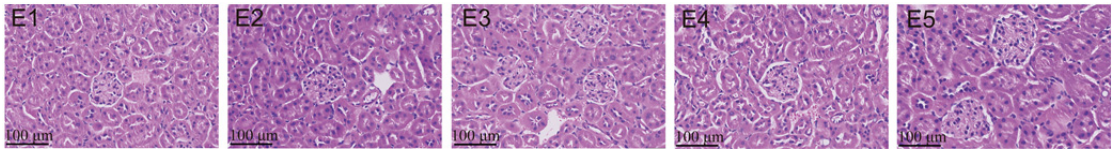

Fig. S2. Effects of *Ficus racemosa* fruit on kidney pathological changes by using H&E staining method; Comparison between the model group (E1), high-dose group (E2), low-dose group (E3), postive group (E4) and control group (E5).

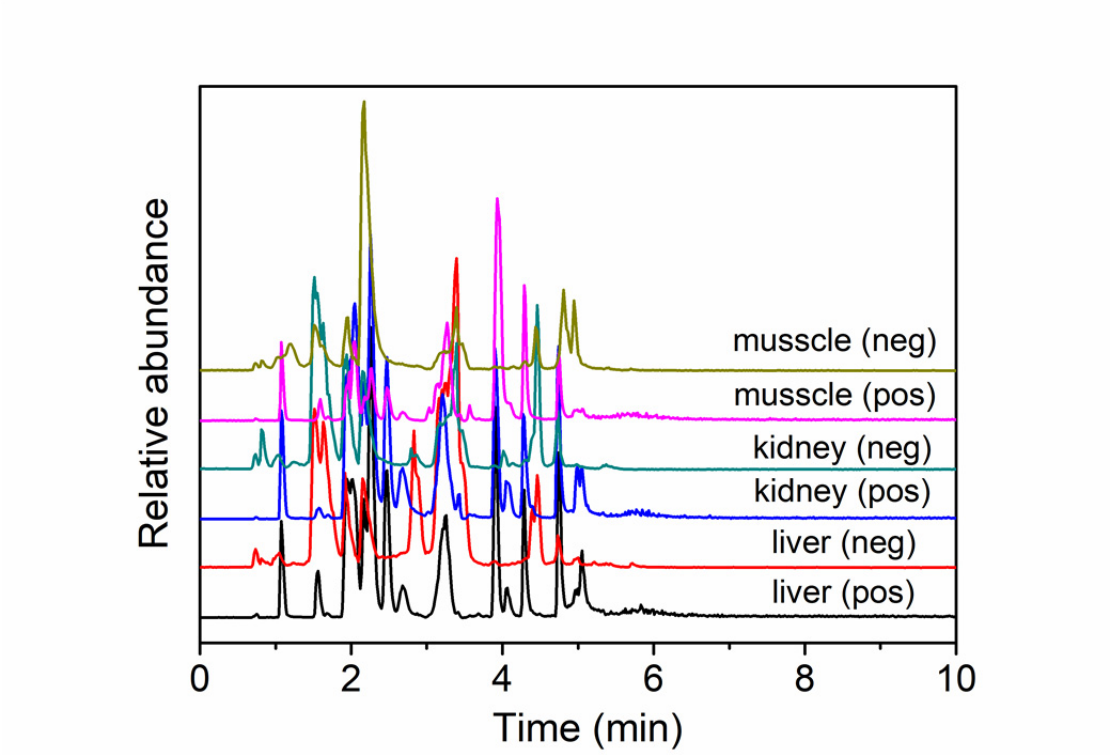

Fig. S3. Typical LC-MS/MS TIC spectra of tissue metabolic profiles. Pos: LC-MS/MS detection with positive mode, neg: LC-MS/MS detection with negative mode.

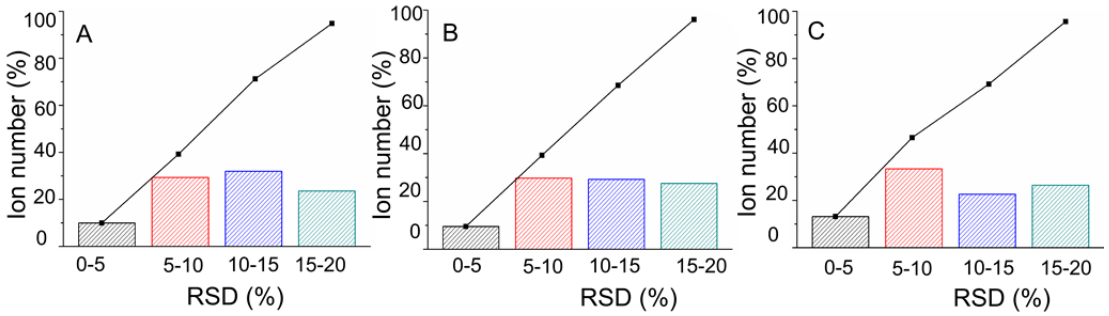

Fig. S4. The percentage of the variables with a relative standard deviation (RSD) below 20% in both ESI positive and negative ion mode in (A) liver, (B) kidney and (C) muscle.

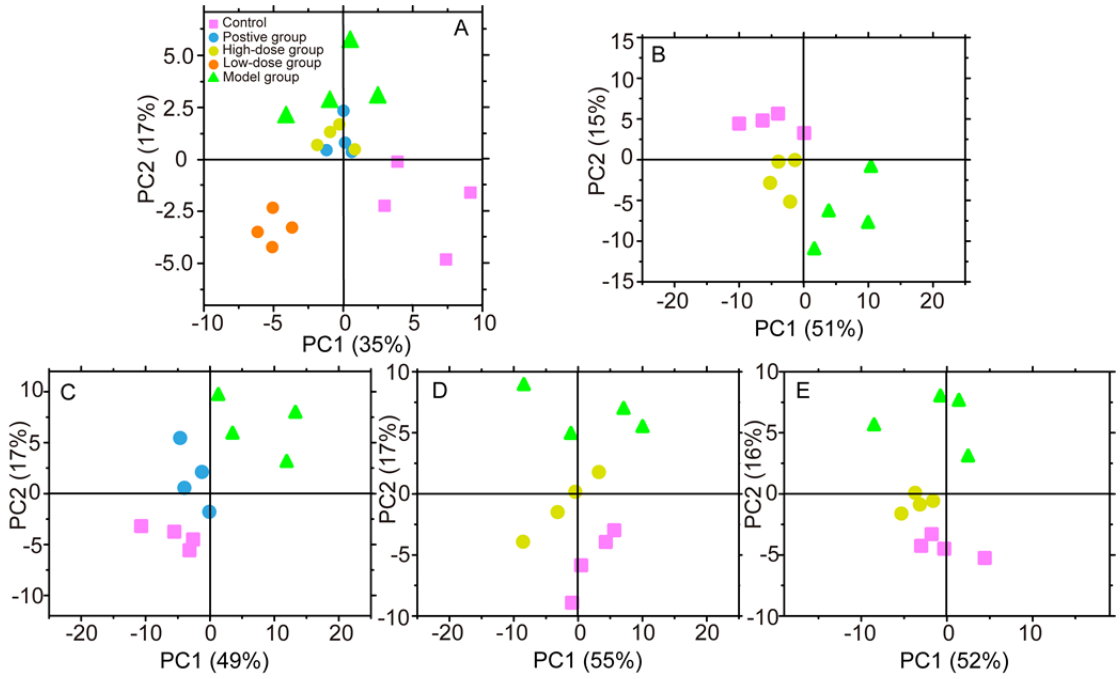

Fig. S5. (A) PCA score plots of PC1 vs. PC2 based on the LC-MS/MS data of liver, (B-E) PLS-DA score plots of PC1 vs. PC2 based on the LC-MS/MS data of liver, kidney and muscle, (B) Control group, high-dose group and model group in liver, (C) Control group, postive dose group and model group in liver, (D) Control group, high-dose group and model group in kidney, (E) Control group, high-dose group and model group in muscle.

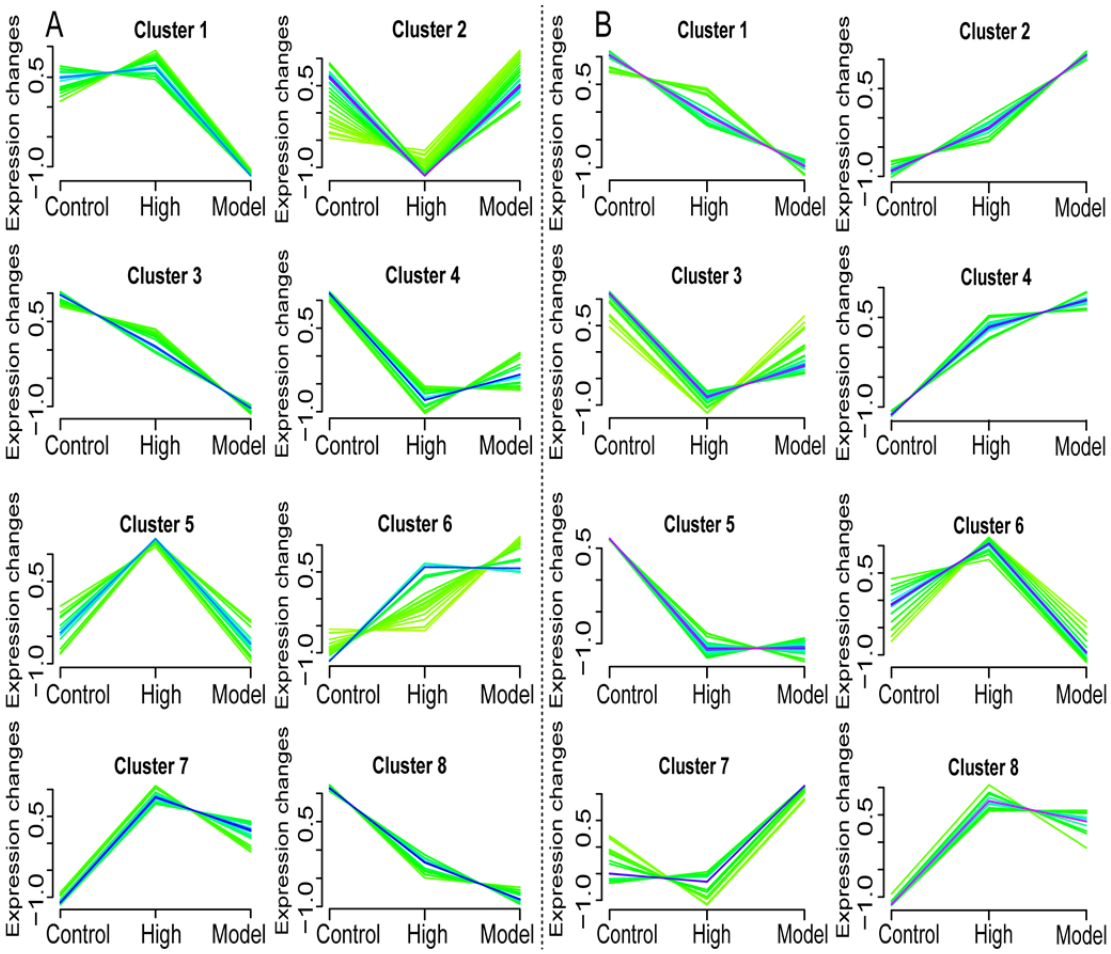

Fig. S6. Identification of specific clusters of metabolites in kidney (A) and muscle (B) using mFuzz analysis. Profiles are represented by metabolite expression changes and the high-dose group, model group, control group.

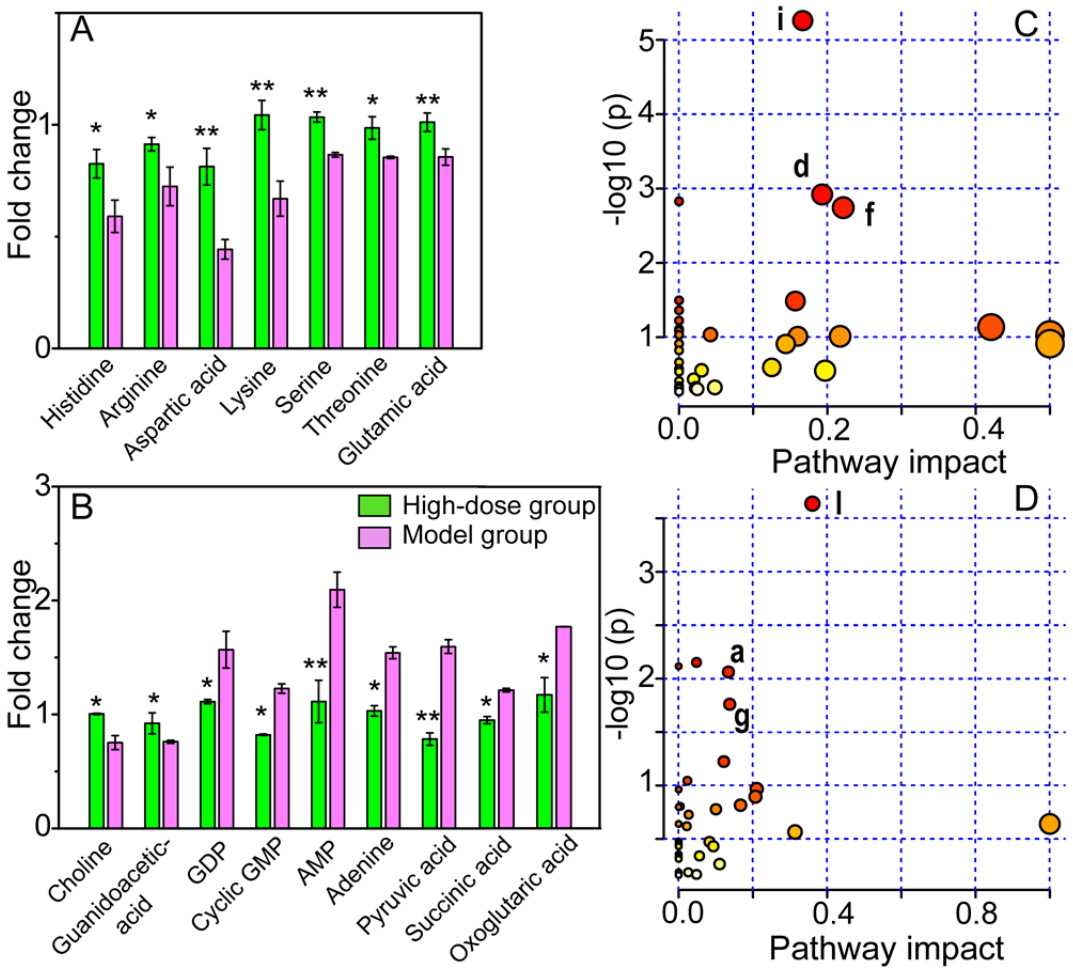

Fig. S7. (A) Fold change of various representative metabolites in model group and high-dose group compared with control group in the kidney, (B) Fold change of various representative metabolites in model group and high-dose group compared with control group in the muscle, (C) The pathway analysis of metabolites in model group, high-dose group and control group in kidney. The node color is based on its p-value, and the node radius is determined based on their pathway impact values, (i) aminoacyl-tRNA biosynthesis; (d) arginine biosynthesis; (f) histidine metabolism, (D) The pathway analysis of metabolites in model group, high-dose group and control group in muscle, (l) glycine, serine and threonine metabolism; (a) purine metabolism; (g) TCA cycle. *$P < 0.05$, **$P < 0.01$, significantly different to model group.

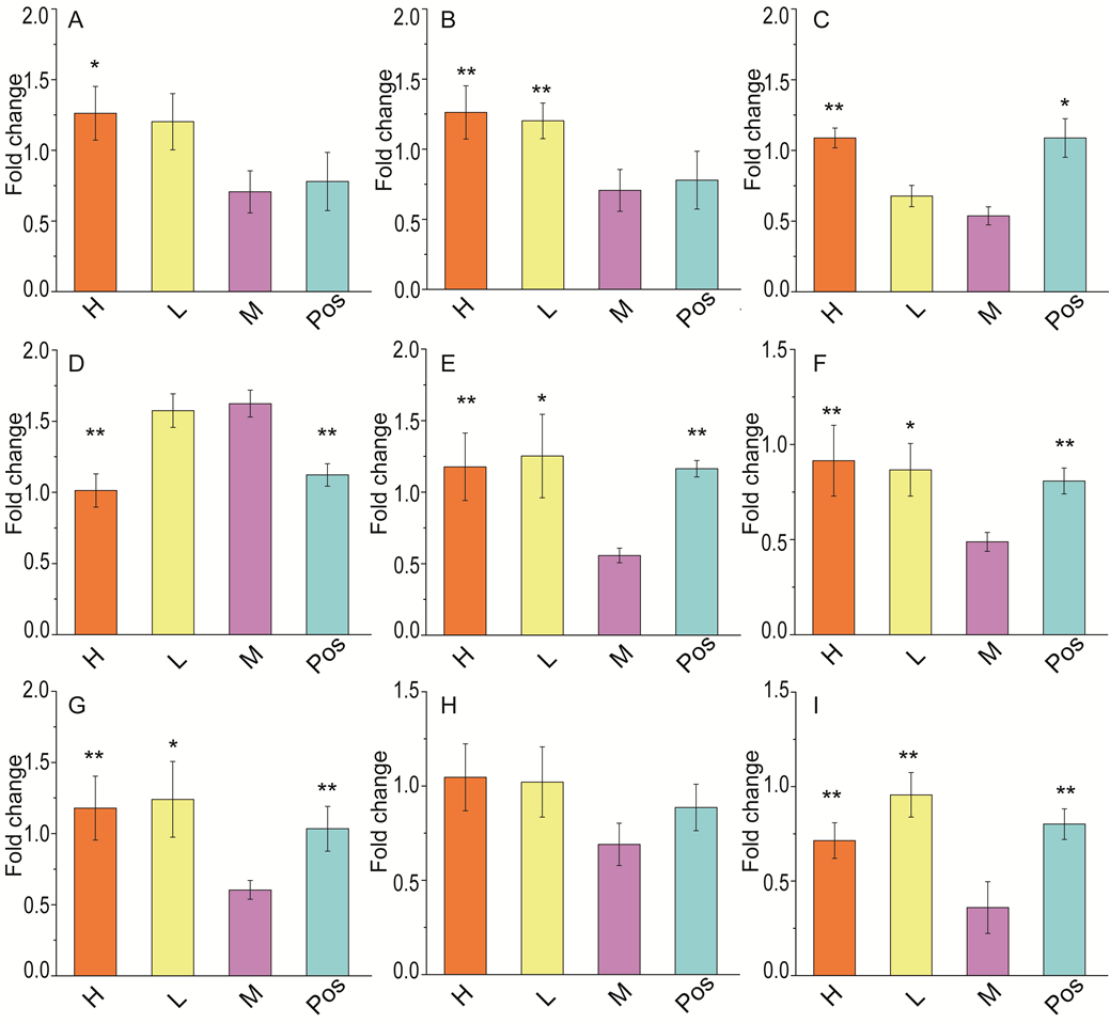

Fig. S8. Fold change of various representative metabolites in high-dose, low-dose, model and postive group compared with control group in the plasma. (A) acetylcholine; (B) asparagine; (C) choline; (D) creatine; (E) glutamic acid; (F) histidine; (G) methyhistamine; (H) serine; (I) threonine.

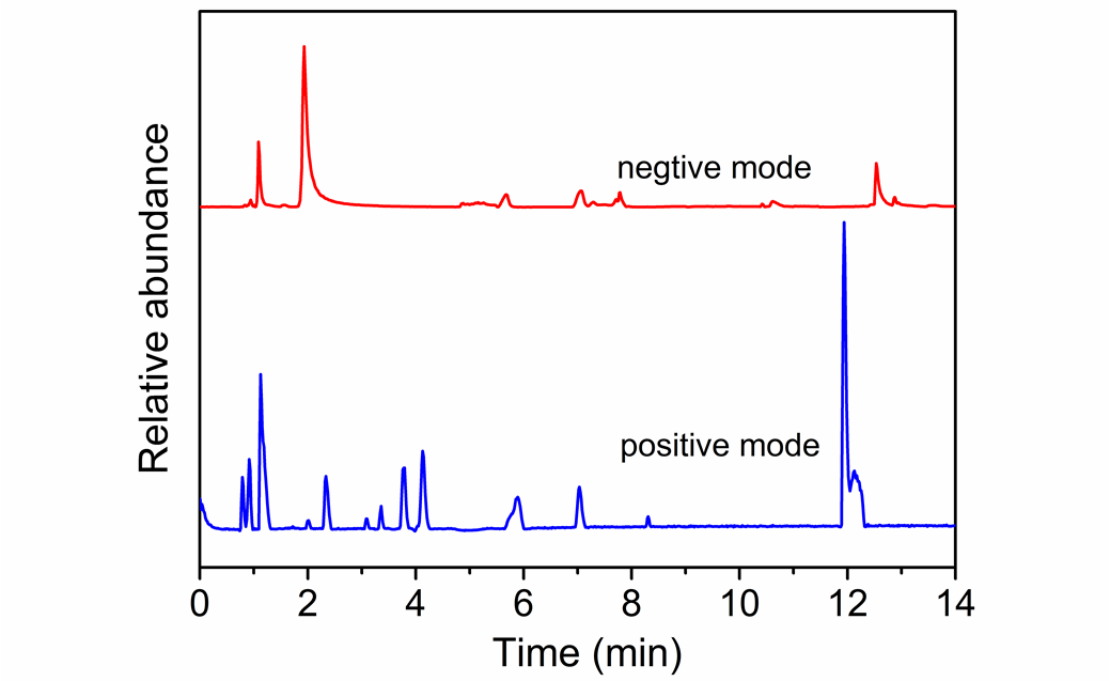

Fig. S9. Typical LC-MS/MS TIC spectra of plasma metabolic profiles. Positive mode: LC-MS/MS detection with positive mode, negative mode: LC-MS/MS detection with negative mode.

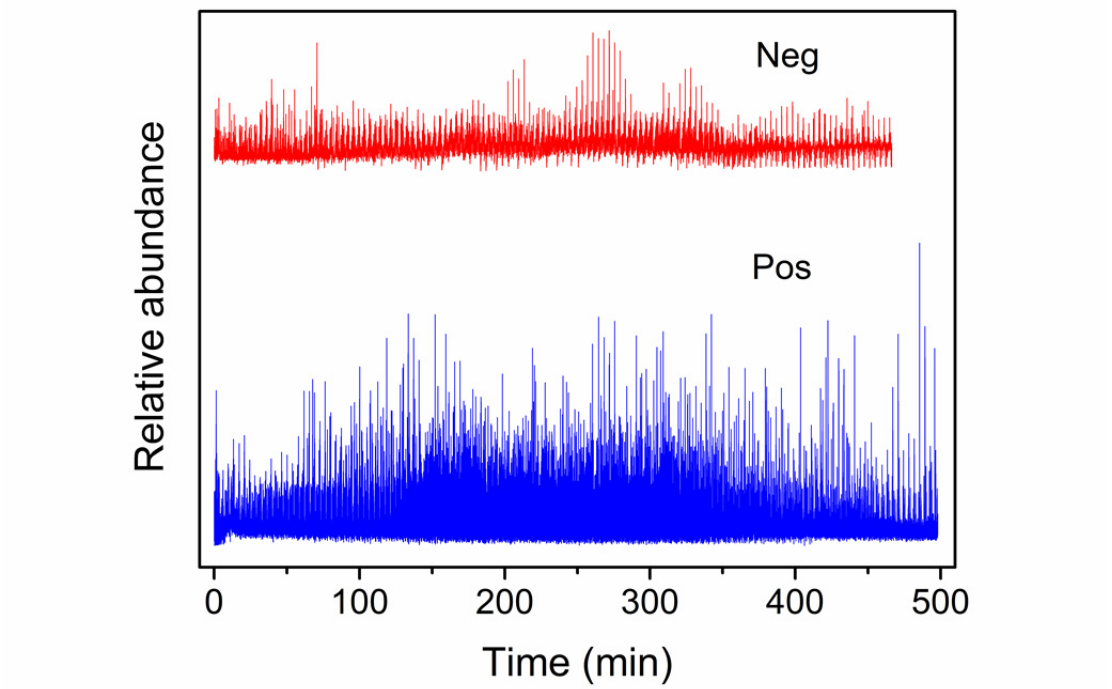

Fig. S10. Typical DESI-MSI TIC spectra of liver sections including model, positive, high-dose, low-dose and control group. Pos: detection with positive mode, neg: detection with negative mode.

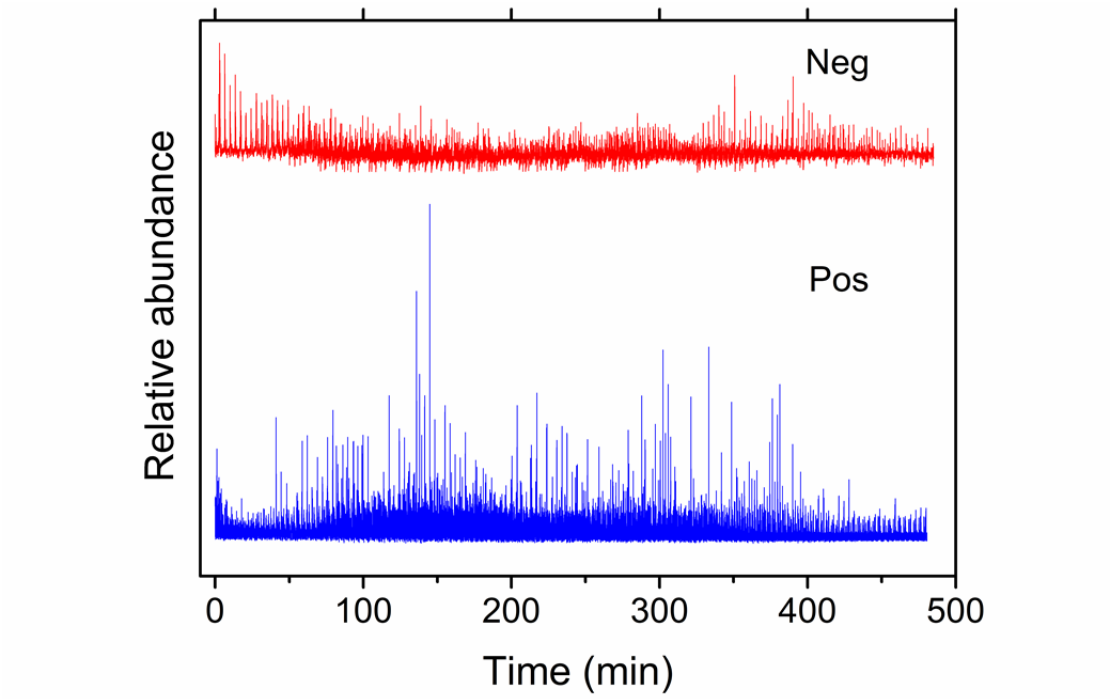

Fig. S11. Typical DESI-MSI TIC spectra of kidney sections including model, positive, high-dose, low-dose and control group. Pos: detection with positive mode, neg: detection with negative mode.

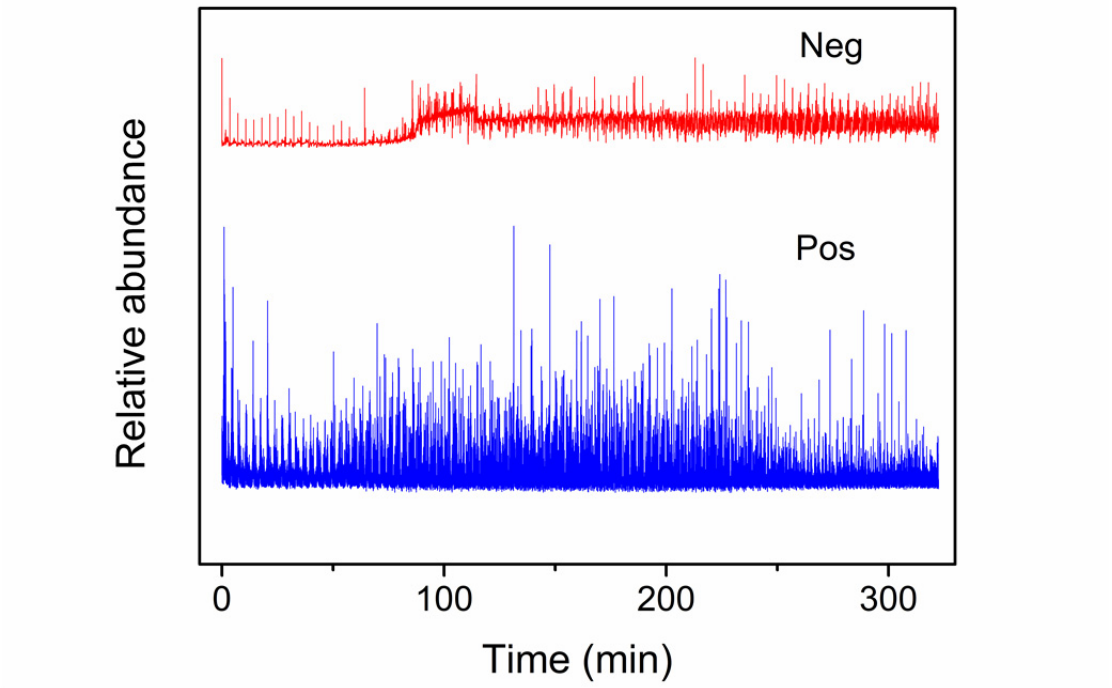

Fig. S12. Typical DESI-MSI TIC spectra of muscle sections including model, positive, high-dose, low-dose and control group. Pos: detection with positive mode, neg: detection with negative mode.

Table S1 Effects of *Ficus racemosa* fruit on FBG, PPG, food consumption, body weight and state of diabetic mice

Treatment	FBG				PPG				Food consumption (g/day/mice)				Body weight				State			
	day 4	day 8	day 12	day 16	day 8		day 16		day 4	day 8	day 12	day 16	day 4	day 8	day 12	day 16	day 4	day 8	day 12	day 16
					1 h	1.5 h	1 h	1.5 h												
control (n=4)	6.3± 0.1	6.5± 0.2	6.3± 0.3	6.4± 0.2	12.5± 0.5	6.9± 0.8	11.3 ±0.6	6.7± 0.5	1.8	2.3	2.3	1.6	22.5 ±0.8	22.9 ±1.2	22.8 ±0.5	23.8 ±0.7	N	N	N	N
model control (n=3)	21.5 ±1.1	22.0 ±1.2	21.8 ±0.5	22.2 ±0.5	29.8± 1.1	27.2 ±0.6	29.9 ±0.9	27.6 ±0.9	4.5	5.0	5.4	4.9	23.0 ±1.1	22.8 ±0.8	22.2 ±0.5	21.3 ±0.4	N	N	N	N
0.6 g/kg/day (n=3)	20.5 ±1.2	19.9 ±1.1	-	-	26.9± 1.0	23.2 ±0.9	-	-	4.1	3.6	1.9	1.3	23.5 ±1.1	21.0 ±1.1	-	-	N	N	B	B*
0.4 g/kg/day (n=3)	22.4 ±1.4	21.9 ±1.0	20.6 ±1.0	19.9 ±0.6	27.2± 0.9	22.9 ±0.8	25.1 ±1.0	22.4 ±1.1	4.7	4.8	3.4	3.2	21.8 ±1.2	21.5 ±0.9	21.8 ±0.5	21.2 ±1.1	N	N	N	N
0.2 g/kg/day (n=3)	21.0 ±1.5	21.1 ±0.3	20.4 ±0.5	20.5 ±0.5	27.0± 0.7	23.5 ±0.6	26.9 ±0.8	23.2 ±0.2	3.6	4.8	4.6	4.1	23.0 ±1.1	22.8 ±0.7	22.5 ±1.5	22.7 ±0.5	N	N	N	N
10 mg/kg/day acarbose (n=3)	21.8 ±0.8	21.9 ±1.0	21.6 ±1.3	20.9 ±0.6	25.2± 0.5	23.9 ±0.7	25.1 ±1.0	22.6 ±1.1	4.3	5.0	5.0	5.2	22.5 ±1.2	21.3 ±0.9	21.8 ±0.8	21.8 ±0.5	N	N	N	N

-:can not be dectected due to the bad state of the mice samples; N: normal; B: bad state and poor appetite; B*: bad state, poor appetite and one death.

Table S2 Summary of liver metabolites treated with *Ficus racemosa* friut and theirs belongings in Mfuzz analysis

Metabolites	Cluster
1-Methylhistamine	1
Glutamic acid	1
Epinephrine	1
Hydroxykynurenine	1
Indoleacetic acid	1
Histidine	1
Isoleucine	1
Spermidine	1
Tyramine	1
13-hydroxyoctadecadienoic acid	1
2-Hydroxyglutarate	1
Adenosine monophosphate	1
Linolenic acid	1
Aminoadipic acid	1
Ascorbic acid	1
Glyceraldehyde-3-phosphate	1
DHAP	1
Hippuric acid	1
Cystathionine	1
Linoleic acid	1
Prostaglandin E2	1
Trans-Aconitic acid	1
1-Methylguanosine	2
Choline	2
Cysteinylglycine	2
Cytosine	2
Acetylcarnitine	2
Cystine	2
Glutamine	2
Melatonin	2
N^6-Methyladenosine	2
Niacinamide	2
Pyroglutamic acid	2
Trimethylamine N-oxide	2
Adenine	2
ADP	2
Allantoin	2
Deoxyuridine triphosphate	2
Erythrose	2
Geranyl-PP	2

Hypoxanthine	2
Indoxyl sulfate	2
Myo-Inositol	2
OMP	2
Oxoglutaric acid	2
Propionic acid	2
Ureidopropionic acid	2
8-Hydroxyguanosine	3
Adenosine	3
Creatine	3
Creatinine	3
Guanosine	3
Inosine	3
Taurine	3
2-Hydroxy-3-methylbutyric acid	3
Alpha-D-Glucose-1,6-bisphosphate	3
Arachidonic.acid	3
Cyclic.GMP	3
Fructose	3
Glucose	3
Fructose 1,6-bisphosphate	3
Fructose 2,6-bisphosphate	3
Fumaric acid	3
Glyceraldehyde	3
Guanosine diphosphate	3
Lactic acid	3
Oxalic acid	3
Phosphoenolpyruvic acid	3
Xanthosine	3
4-Hydroxyproline	4
5-Aminopentanoic acid	4
Acetylcholine	4
Indolelactic acid	4
Aspartic acid	4
Homoserine	4
Kynurenine	4
Serine	4
Threonine	4
Valine	4
Uridine	4
2-Phosphoglyceric acid	4
3-Phosphoglyceric acid	4
Lactose	4
Azelaic acid	4

Chenodeoxycholic acid	4
Chenodeoxycholic acid glycine conjugate	4
Glucose-1-phosphate	4
Glyceric acid	4
Heptadecanoic acid	4
Inosinic acid	4
2-Aminobenzoic.acid	5
5-Aminolevulinic.acid	5
Dimethylglycine	5
Phenylalanine	5
Tyrosine	5
Serotonin	5
3-Methyl-2-oxovaleric acid	5
3-Nitrotyrosine	5
Mevalonic acid	5
N-Acetylneuraminic acid	5
Orotic acid	5
Pyruvic acid	5
Uracil	5
3-Aminoisobutanoic acid	6
4-Trimethylammoniobutanoic acid	6
5-Hydroxymethyl-2-deoxyuridine	6
Cadaverine	6
cGAMP	6
Ornithine	6
Indole	6
Kynurenic acid	6
Arginine	6
Cysteine	6
Lysine	6
2-Dimethylguanosine	6
3-Hydroxybutyric acid	6
Acetoacetic acid	6
Acetylglycine	6
Adenylsuccinic acid	6
Cytidine monophosphate	6
Glucuronic acid	6
Ribose-5-phosphate	6
Glycocholic acid	6
Malondialdehyde	6
Malonic acid	6
Pyridoxal 5’-phosphate	6
Taurocholic acid	6
4-Hydroxybutyric acid	7

Indolepyruvate	7
Asparagine	7
Methionine	7
4-Pyridoxic acid	7
Adipic acid	7
Benzoic acid	7
Biotin	7
Citraconic acid	7
Leucic acid	7
Fructose-1-phosphate	7
Glutaric acid	7
Glutathione	7
Guanidoacetic acid	7
Malic acid	7
Maleic acid	7
Methylsuccinic acid	7
Oxalacetic acid	7
Oxidized glutathione	7
Pantothenic acid	7
Quinolinic acid	7
Alanine	8
Carnitine	8
Normetanephrine	8
Pipecolic acid	8
Spermine	8
Tryptamine	8
5-Thymidylic acid	8
Adenosine triphosphate	8
Citrulline	8
Glycerol-3-phosphate	8
Homogentisic acid	8
Methylmalonic acid	8
Myristic acid	8
Oxypurinol	8
Phosphoribosyl pyrophosphate	8
Succinic acid	8
Uric acid	8
Nicotinic acid	8

Table S3 Metabolite pathway changes in liver treated with *Ficus racemosa* fruit

No.	Pathway name	Match Status[a]	p	-log(p)	Impact	Hits[b]
a	Purine metabolism	10/65	1.03E-4	3.99	0.13	Phosphoribosyl pyrophosphate, Guanosine diphosphate(GDP), Cyclic GMP, Guanosine, Adenosine triphosphate(ATP), Adenosine, Inosine, Uric acid, Xanthosine, Adenosine monophosphate
b	Glycolysis / Gluconeogenesis	5/26	0.002	2.63	0.26	Lactic acid, Phosphoenolpyruvic acid, Fructose 1,6-bisphosphate, Glyceraldehyde-3-phosphate, DHAP
c	Fructose and mannose metabolism	4/20	0.006	2.24	0.09	Fructose 2,6-bisphosphate, Glyceraldehyde 3-phosphate, Fructose 1,6-bisphosphate, Glycerone phosphate
d	Arginine biosynthesis	3/14	0.014	1.84	0.35	Citrulline, Fumaric acid, Glutamic acid
e	Alanine, aspartate and glutamate metabolism	4/28	0.019	1.71	0.20	Fumaric acid, Alanine, Succinic acid, Glutamic acid
f	Histidine metabolism	3/16	0.021	1.69	0.31	Histidine, 1-Methylhistamine, Glutamic acid
g	TCA cycle	3/20	0.038	1.42	0.06	Succinic acid, Fumaric acid, Phosphoenolpyruvic acid
h	beta-Alanine metabolism	3/21	0.043	1.37	0.06	Spermine, Histidine, Spermidine

a, the matched number of metabolites in one pathway; b, the matched metabolites in one pathway.

Table S4 Liver metabolites included in clusters 1, 2, 6 and 8 in Mfuzz analysis treated with acarbose

Metabolites	RT	Molecular weight	Cluster
8-Hydroxy-deoxyguanosine	4.797	283.15	1
Hydroxykynurenine	**0.819**	**224.15**	**1**
Indoleacetic acid	**4.819**	**175.1**	**1**
Kynurenic acid	1.620	189.0	1
Kynurenine	2.242	208.1	1
Uridine	1.594	244.15	1
13-hydroxyoctadecadienoic acid	**0.752**	**296.2**	**1**
4-Hydroxyphenylpyruvic acid	1.831	180.0	1
Acetoacetic acid	1.801	102.0	1
Linolenic acid	**0.735**	**280.1**	**1**
Aminoadipic acid	**4.710**	**161.1**	**1**
Biotin	1.566	244.1	1
Chenodeoxycholic acid	4.635	392.0	1
Oxalacetic acid	4.395	132.0	1
Taurocholic acid	1.307	515.5	1
8-Hydroxyguanosine	**2.900**	**299.15**	**2**
Adenosine	**1.564**	**267.15**	**2**
Cytosine	2.238	111.1	2
Alanine	**3.914**	**89.0**	**2**
Carnitine	**3.923**	**161.1**	**2**
Cystine	2.322	240.1	2
Glutamine	4.296	146.1	2
Niacinamide	1.084	122.0	2
Pipecolic acid	**3.289**	**129.0**	**2**
Pyroglutamic acid	3.157	129.0	2
Spermine	**4.130**	**202.1**	**2**
Tryptamine	**1.675**	**160.1**	**2**
2-Hydroxy-3-methylbutyric acid	**1.434**	**118.0**	**2**
5-Thymidylic acid	**0.782**	**322.1**	**2**
Adenine	1.541	135.1	2
Adenosine triphosphate	**0.870**	**506.9**	**2**
Adenylsuccinic acid	0.896	463.1	2
Citrulline	**4.439**	**175.0**	**2**
Cyclic GMP	**0.932**	**345.0**	**2**
Glyceraldehyde	**2.900**	**90.0**	**2**
Glycerol-3-phosphate	**5.146**	**172.0**	**2**
Homogentisic acid	**0.989**	**168.0**	**2**
Indoxyl sulfate	0.855	213.1	2
Lactic acid	**2.191**	**90.0**	**2**
Methylmalonic acid	**1.446**	**118.0**	**2**

Oxalic acid	**2.190**	**90.0**	**2**
Oxoglutaric acid	4.284	146.0	2
Propionic acid	1.510	74.0	2
1-Methylhistamine	**2.315**	**125.0**	**6**
4-Trimethylammoniobutanoic acid	4.308	146.1	6
Acetylcholine	4.106	145.1	6
Ornithine	5.099	132.1	6
Indolepyruvate	4.678	203.1	6
Homoserine	4.066	119.1	6
Threonine	4.065	119.101	6
Tyramine	**2.921**	**137.1**	**6**
Adipic acid	2.051	145.9	6
Alpha-ketoisovaleric acid	3.362	116.1	6
Azelaic acid	1.050	188.0	6
Glucuronic acid	4.728	194.0	6
DHAP	**4.681**	**169.9**	**6**
Leucic acid	1.256	132.0	6
Glyceric acid	4.328	106.0	6
Linoleic acid	**0.738**	**278.1**	**6**
Malic acid	4.750	134.0	6
Maleic acid	1.040	116.0	6
Quinolinic acid	1.043	167.0	6
Trans-Aconitic acid	**2.816**	**174.0**	**6**
4-Hydroxyproline	2.272	131.1	8
Glutamic acid	**4.758**	**147.1**	**8**
Arginine	4.998	174.1	8
Asparagine	4.405	132.1	8
Histidine	**4.987**	**155.1**	**8**
Isoleucine	**2.479**	**131.1**	**8**
Serine	4.344	105.0	8
Spermidine	**6.096**	**145.1**	**8**
2-Hydroxyglutarate	**4.743**	**148.0**	**8**
2-Phosphoglyceric acid	1.101	186.0	8
3-Phosphoglyceric acid	0.996	186.0	8
Glutaric acid	2.442	132.0	8
Guanidoacetic acid	4.031	117.0	8
Hippuric acid	**1.471**	**179.0**	**8**
Methylsuccinic acid	2.424	132.0	8
Pyridoxal 5’-phosphate	5.022	247	8

Bold: Metabolites changes were also found when treated with *Ficus racemosa* fruit.

Table S5 Metabolite pathway changes in liver treated with acarbose

No.	Pathway name	Match Status[a]	p	-log(p)	Impact	Hits[b]
i	Aminoacyl-tRNA biosynthesis	8/48	0.001	2.868	0.17	Asparagine, Histidine, Arginine, Serine, Alanine, Isoleucine, Threonine, Glutamate
e	Alanine, aspartate and glutamate metabolism	6/28	0.001	2.83	0.36	Asparagine, N6-(1,2-Dicarboxyethyl)-AMP, Alanine, Glutamate, Oxaloacetate, 2-Oxoglutarate
d	Arginine biosynthesis	4/14	0.003	2.50	0.42	Glutamate, Arginine, Citrulline, 2-Oxoglutarate
j	Butanoate metabolism	4/15	0.004	2.38	0.11	Acetoacetate, Glutamate, 2-Oxoglutarate, 2-Hydroxyglutarate
k	Glyoxylate and dicarboxylate metabolism	5/32	0.015	1.82	0.15	Malate, Serine, Glutamate, D-Glycerate, Oxaloacetate
l	Glycine, serine and threonine metabolism	5/33	0.017	1.77	0.27	Serine, Guanidinoacetate, 3-Phospho-D-glycerat, Threonine, D-Glycerate
m	Lysine degradation	4/25	0.027	1.57	0.15	4-Trimethylammoniobutanoate, L-2-Aminoadipate, L-Pipecolate, Carnitine
n	D-Glutamine and D-glutamate metabolism	2/6	0.029	1.54	0.50	Glutamate, 2-Oxoglutarate
o	Arginine and proline metabolism	5/38	0.030	1.52	0.20	Arginine, Guanidinoacetate, Spermidine, Spermine, Glutamate
b	Glycolysis / Gluconeogenesis	4/26	0.031	1.51	0.04	Lactate, 3-Phospho-D-glycerat, Oxaloacetate, Glycerone phosphate

p	Glycerolipid metabolism	3/16	0.036	1.44	0.14	sn-Glycerol 3-phosphate, D-Glycerate, Glycerone phosphate
f	Histidine metabolism	3/16	0.036	1.44	0.31	Glutamate, Histidine, N-Methylhistamine
q	Glutathione metabolism	4/28	0.039	1.41	0.03	Glutamate, 5-Oxoproline, Spermidine, Spermine
r	Tryptophan metabolism	5/41	0.04	1.40	0.15	5-Hydroxyindoleacetate, L-Kynurenine, Tryptamine, Indolepyruvate, Indole-3-acetate

a, the matched number of metabolites in one pathway; b, the matched metabolites in one pathway.

Table S6 Summary of liver metabolites treated with acarbose and theirs belongings in Mfuzz analysis

Metabolites	Cluster
8-Hydroxy deoxyguanosine	1
Hydroxykynurenine	1
Indoleacetic acid	1
Kynurenic acid	1
Kynurenine	1
Uridine	1
13-hydroxyoctadecadienoic acid	1
4-Hydroxyphenylpyruvic acid	1
Acetoacetic acid	1
Linolenic acid	1
Aminoadipic acid	1
Biotin	1
Chenodeoxycholic acid	1
Oxalacetic acid	1
Taurocholic acid	1
8-Hydroxyguanosine	2
Adenosine	2
Cytosine	2
Alanine	2
Carnitine	2
Cystine	2
Glutamine	2
Niacinamide	2
Pipecolic acid	2
Pyroglutamic acid	2
Spermine	2
Tryptamine	2
2-Hydroxy-3-methylbutyric acid	2
5-Thymidylic acid	2
Adenine	2
Adenosine triphosphate	2
Adenylsuccinic acid	2
Citrulline	2
Cyclic GMP	2
Glyceraldehyde	2
Glycerol-3-phosphate	2
Homogentisic acid	2
Indoxyl sulfate	2
Lactic acid	2
Methylmalonic acid	2

Oxalic acid	2
Oxoglutaric acid	2
Propionic acid	2
2-Aminobenzoic acid	3
3-Aminoisobutanoic acid	3
5-Aminolevulinic acid	3
5-Hydroxy-L-tryptophan	3
Serotonin	3
Adenosine monophosphate	3
Allantoin	3
Inosinic acid	3
Mevalonic acid	3
Orotic acid	3
Pyruvic acid	3
Uracil	3
Nicotinic acid	3
2-Aminobenzoic acid	4
4-Hydroxybutyric acid	4
5-Aminopentanoic acid	4
Dimethylglycine	4
Epinephrine	4
Indolelactic acid	4
Aspartic acid	4
Methionine	4
Tryptophan	4
Valine	4
N2-Dimethylguanosine	4
3-Methyl-2-oxovaleric acid	4
4-Pyridoxic acid	4
Lactose	4
Ascorbic acid	4
Benzoic acid	4
Chenodeoxycholic acid glycine conjugate	4
Citraconic acid	4
Glyceraldehyde-3-phosphate	4
Fructose-1-phosphate	4
Glucose-1-phosphate	4
Glutathione	4
Heptadecanoic acid	4
Cystathionine	4
N-Acetylneuraminic acid	4
Oxidized glutathione	4
Pantothenic acid	4
Prostaglandin E2	4

Choline	5
Guanosine	5
Inosine	5
Cysteine	5
Melatonin	5
Normetanephrine	5
Taurine	5
Arachidonic acid	5
Deoxyuridine triphosphate	5
Glucose	5
Guanosine diphosphate	5
Galactose	5
OMP	5
Phosphoenolpyruvic acid	5
1-Methylhistamine	6
4-Trimethylammoniobutanoic acid	6
Acetylcholine	6
Ornithine	6
Indolepyruvate	6
Homoserine	6
Threonine	6
Tyramine	6
Adipic acid	6
Alpha-ketoisovaleric acid	6
Azelaic acid	6
Glucuronic acid	6
DHAP	6
Leucic acid	6
Glyceric acid	6
Linoleic acid	6
Malic acid	6
Maleic acid	6
Quinolinic acid	6
Trans-Aconitic acid	6
1-Methyladenosine	7
1-Methylguanosine	7
5-Hydroxymethyl-2-deoxyuridine	7
cGAMP	7
Creatine	7
Creatinine	7
Cysteinylglycine	7
Deoxyuridine	7
Glucosamine	7
Indole	7

Acetylcarnitine	7
Lysine	7
N6-Methyladenosine	7
Trimethylamine-N-oxide	7
3-Hydroxybutyric acid	7
Acetylglycine	7
ADP	7
Alpha-D-Glucose-1,6-bisphosphate	7
Cytidine monophosphate	7
Erythrose	7
Fructose 1,6-bisphosphate.	7
Fructose 2,6-bisphosphate	7
Fumaric acid	7
Geranyl-PP	7
Glycocholic acid	7
Hypoxanthine	7
Malonic acid	7
Myo-Inositol	7
Myristic acid	7
Phosphoribosyl pyrophosphate	7
Succinic acid	7
Ureidopropionic acid	7
Uric acid	7
Xanthosine	7
4-Hydroxyproline	8
Glutamic acid	8
Arginine	8
Asparagine	8
Histidine	8
Isoleucine	8
Serine	8
Spermidine	8
2-Hydroxyglutarate	8
2-Phosphoglyceric acid	8
3-Phosphoglyceric acid	8
Glutaric acid	8
Guanidoacetic acid	8
Hippuric acid	8
Methylsuccinic acid	8
Pyridoxal 5’-phosphate	8

Table S7 Summary of kidney metabolites treated with *Ficus racemosa* friut and theirs belongings in Mfuzz analysis

Metabolites	Cluster
Glutamic acid	1
Homocysteine	1
Arginine	1
Aspartic acid	1
Carnitine	1
Lysine	1
Serine	1
3-Methyl-2-oxovaleric acid	1
Adenosine monophosphate	1
Glucuronic acid	1
Hippuric acid	1
Inosinic acid	1
Orotic acid	1
Creatine	2
Cytidine	2
Mannose	2
Hydroxykynurenine	2
Inosine	2
Cystine	2
Tyrosine	2
Melatonin	2
N2-Dimethylguanosine	2
N-Acetylputrescine	2
Normetanephrine	2
Salicyluric acid	2
Spermine	2
Uridine	2
13-hydroxyoctadecadienoic acid	2
3-Nitrotyrosine	2
4-Hydroxyphenylpyruvic acid	2
Adenylsuccinic acid	2
ADP	2
Allantoin	2
D-glucose 1,6-bisphosphate	2
Arachidonic acid	2
Citrulline	2
Cytidine monophosphate	2
Fructose 1,6-bisphosphate.	2
Fructose 2,6-bisphosphate	2
Homogentisic acid	2
Linoleic acid	2

Maleic acid	2
Propionic acid	2
Uracil	2
8-Hydroxy deoxyguanosine	3
Indolepyruvate	3
Histidine	3
Threonine	3
Tyramine	3
2-Hydroxyglutarate	3
3-Hydroxybutyric acid	3
Acetylglycine	3
Fructose 1-phosphate	3
Fructose 6-phosphate	3
Glucose 6-phosphate	3
Cystathionine	3
Methylmalonic acid	3
Myristic acid	3
1-Methylguanosine	4
2-Aminobenzoic acid	4
5-Aminolevulinic acid	4
Dimethylglycine	4
Glycine	4
Indole	4
Kynurenine	4
Tryptophan	4
Spermidine	4
Xanthurenic acid	4
12-HETE	4
Adipic acid	4
Linolenic acid	4
Azelaic acid	4
Biotin	4
Glyceraldehyde-3-phosphate	4
Glyceric acid	4
Heptadecanoic acid	4
Hypoxanthine	4
Pantothenic acid	4
1-Methyladenosine	5
1-Methylhistamine	5
4-Trimethylammoniobutanoic acid	5
Acetylcholine	5
Cadaverine	5
Homoserine	5
Serotonin	5

Adenosine triphosphate	5
Ribose 5-phosphate	5
Guanosine diphosphate	5
Phosphoribosyl pyrophosphate	5
Pyridoxal 5’-phosphate	5
Succinic acid	5
Uric acid	5
8-Hydroxyguanosine	6
Adenosine	6
Betaine	6
Cytosine	6
Epinephrine	6
Guanosine	6
Histamine	6
Proline	6
Pipecolic acid	6
Pyroglutamic acid	6
Trimethylamine N-oxide	6
2-Phosphoglyceric acid	6
3-Phosphoglyceric acid	6
Adenine	6
5-Thymidylic acid	6
Cyclic GMP	6
Fumaric acid	6
Ascorbic acid	6
Benzoic acid	6
Citraconic acid	6
Erythrose	6
Galactose	6
Myo-Inositol	6
OMP	6
Xanthosine	6
N-Acetylneuraminic acid	6
Prostaglandin E2	6
Xanthine	6
4-Hydroxyproline	7
5-Aminopentanoic acid	7
Alanine	7
Asparagine	7
Cysteine	7
Isoleucine	7
Leucine	7
N6-Methyladenosine	7
Niacinamide	7

Sarcosine	7
Taurine	7
Tryptamine	7
4-Pyridoxic acid	7
Lactose	7
Glucose	7
Leucic acid	7
Glycerol 3-phosphate	7
Indoxyl sulfate	7
Lactic acid	7
Oxalic acid	7
Oxypurinol	7
Phosphoenolpyruvic acid	7
Pyruvic acid	7
Ureidopropionic acid	7
3-Methylhistidine	8
Ornithine	8
Glucosamine	8
Aminoadipic acid	8
DHAP	8
Glutaric acid	8
Guanidoacetic acid	8
Homovanillic acid	8
Isovaleric acid	8
Malonic acid	8
Methylsuccinic acid	8
Mevalonic acid	8
Quinolinic acid	8
Taurocholic acid	8
Trans-Aconitic acid	8

Table S8 Summary of muscle metabolites treated with *Ficus racemosa* fruit and theirs belongings in Mfuzz analysis

Metabolites	Cluster
1-Methylguanosine	1
3-Aminoisobutanoic acid	1
Choline	1
Hydroxykynurenine	1
Tryptophan	1
Melatonin Melatonin	1
4-Hydroxyphenylpyruvic acid	1
4-Pyridoxic acid	1
ADP	1
Allopurinol	1
Arachidonic acid	1
Guanidoacetic acid	1
Isovaleric acid	1
Oxypurinol	1
Prostaglandin E2	1
Xanthine	1
Creatinine	2
Glutamine	2
Histidine	2
Serine	2
Pipecolic acid	2
2-Hydroxyglutarate	2
Adenine	2
Adenosine monophosphate	2
Lactose	2
Ascorbic acid	2
Lactic acid	2
Oxalic acid	2
Oxoglutaric acid	2
Quinolinic acid	2
2-Aminobenzoic acid	3
Adenosine	3
Betaine	3
Cytosine	3
Ornithine	3
Guanosine monophosphate	3
Tyrosine	3
Nicotinic acid	3
Normetanephrine	3
Pyroglutamic acid	3

Uridine	3
Xanthurenic acid	3
3-Methyl-2-oxovaleric acid	3
5-Thymidylic acid	3
Glucose 1,6-bisphosphate	3
Azelaic acid	3
Citraconic acid	3
Fructose 1,6-bisphosphate	3
Fructose 2,6-bisphosphate	3
Glutaric acid	3
Homovanillic acid	3
Inosinic acid	3
Mevalonic acid	3
Pantothenic acid	3
Propionic acid	3
Glutamic acid	4
Glycine	4
Arginine	4
Serotonin	4
Taurine	4
Trimethylamine N-oxide	4
Citrulline	4
Glucuronic acid	4
DHAP	4
Glycerol 3-phosphate	4
Guanosine triphosphate	4
Maleic acid	4
Oxalacetic acid	4
Cytidine	5
Deoxyuridine	5
Dimethylglycine	5
Mannose	5
Guanosine	5
Histamine	5
Inosine	5
Carnitine	5
Methionine	5
Threonine	5
N-Acetylputrescine	5
13-hydroxyoctadecadienoic acid	5
Linolenic acid	5
Biotin	5
Cytidine monophosphate	5
Fumaric acid	5

Heptadecanoic acid	5
Homogentisic acid	5
Hypoxanthine	5
Linoleic acid	5
Malic acid	5
Methylmalonic acid	5
Myo-Inositol	5
Myristic acid	5
OMP	5
Uracil	5
Uric acid	5
Xanthosine	5
4-Hydroxyproline	6
Epinephrine	6
Indole	6
Indolelactic acid	6
Kynurenine	6
Tyramine	6
3-Hydroxybutyric acid	6
Acetylglycine	6
Adenosine triphosphate	6
Adenylsuccinic acid	6
Hippuric acid	6
Asparagine	6
Indoxyl sulfate	6
Malonic acid	6
N-Acetylneuraminic acid	6
Taurocholic acid	6
Trans-Aconitic acid	6
Ureidopropionic acid	6
Lysine	6
Proline	6
3-Methylhistidine	7
4-Trimethylammoniobutanoic acid	7
Alanine	7
Sarcosine	7
Spermine	7
Tryptamine	7
2-Hydroxy-3-methylbutyric acid	7
Benzoic acid	7
Cyclic GMP	7
Leucic acid	7
Glyceric acid	7
Guanosine diphosphate	7

Methylsuccinic acid	7
Orotic acid	7
Oxidized glutathione	7
Pyruvic acid	7
Succinic acid	7
1-Methyladenosine	8
8-Hydroxy-deoxyguanosine	8
Aspartic acid	8
Phenylalanine	8
N6-Methyladenosine	8
Adipic acid	8
Chenodeoxycholic acid	8
Glucose	8
Fructose 1-phosphate	8

Table S9 Metabolite pathway changes in kidney treated with *Ficus racemosa* fruit

No.	Pathway name	Match Status[a]	p	-log(p)	Impact	Hits[b]
i	Aminoacyl-tRNA biosynthesis	7/48	5.5047 E-6	5.26	0.17	Histidine, Arginine, Aspartate, Serine, Lysine, Threonine, Glutamate
d	Arginine biosynthesis	3/14	0.001	2.92	0.19	Arginine, Aspartate, Glutamate
f	Histidine metabolism	3/16	0.002	2.74	0.22	Histidine, Glutamate, Aspartate

a, the matched number of metabolites in one pathway; b, the matched metabolites in one pathway.

Table S10 Metabolite pathway changes in muscle treated with *Ficus racemosa* fruit

No.	Pathway name	Match Status[a]	p	-log(p)	Impact	Hits[b]
l	Glycine, serine and threonine metabolism	6/33	2.3033 E-4	3.64	0.36	Serine, Choline, Guanidinoacetate, Sarcosine, D-Glycerate, Pyruvate
a	Purine metabolism	6/65	0.009	2.06	0.13	GDP, Xanthine, ADP, AMP, 3',5'-Cyclic GMP, Adenine
g	TCA cycle	3/20	0.017	1.76	0.14	Succinate, 2-Oxoglutarate, Pyruvate

a, the matched number of metabolites in one pathway; b, the matched metabolites in one pathway.

Table S11 Summary of the representative metabolite variations in different conditions

Compound	RT	Molecular weight	Liver			Kidney		Muscle	
			H-Con	Pos-Con	MC-Con	H-Con	MC-Con	H-Con	MC-Con
Amino acids									
1-Methylhistamine	2.315	125.0	0.3	-0.1	-0.6				
Glutamic acid	4.758	147.1	-0.1	0.0	-0.3	0.0	-0.1		
Alanine	3.914	89.0	0.0	0.0	0.2				
Asparagine	4.405	132.1	-0.4	-0.1	-0.9	-0.3	-0.8		
Histidine	4.987	155.1	-0.1	-0.1	-0.4	-0.2	-0.6		
Serine	4.344	105.0	-0.1	-0.1	-0.3	0.0	-0.1		
Threonine	4.065	119.1	-0.1	0.1	-0.2	0.0	-0.2		
Citrulline	4.439	175.0	0.1	0.1	0.8				
Arginine	4.998	174.1	0.2	0.1	-0.3	-0.1	-0.3		
Lysine	5.059	146.101				-0.0	-0.4		
Organic acids									
Fructose 1,6-bisphosphate	0.736	340.0	0.0	0.5	0.5				
DHAP	4.681	169.9	-0.5	-1.0	-1.4				
Glyceraldehyde-3-phosphate	4.733	169.9	0.1	-0.3	-0.2				
3-Phosphoglyceric acid	0.996	186.0	-0.3	-0.1	-0.4				
Phosphoenolpyruvic acid	4.728	167.9	0.1	0.1	0.4				
Lactate	2.191	90.0	-0.0	-0.1	0.3				
Glycerol-3-phosphate	5.146	172.0	0.3	0.2	0.9				
Succinic acid	2.164	118.0	-0.1	0.4	0.5			0.0	0.2
Fumaric acid	4.470	116.0	0.1	0.3	0.3				
Acetoacetic acid	1.801	102.0	0.3	0.0	-0.2				
Creatine	3.957	131.1	0.0	0.3	0.2				
Homogentisic acid	0.989	168.0	-0.2	-0.1	0.3				
Guanidoacetic acid	4.031	117.0		0.0	-0.2			0.0	-0.3
Pyruvic acid	1.590	88.0						-0.2	0.6
Oxoglutaric acid	4.284	146.0		-0.1	0.4			0.1	0.4
Uric acid	3.718	168.0	-0.1	1.2	1.3				
Carbohydrates									
Glucose	3.353	180.0	0.2	0.3	0.4				
Amines									
Spermine	4.130	202.1	0.1	0.0	0.5				
Alkalines									
Choline	2.041	103.1						0.0	-0.3
Adenine	1.541	135.1						0.0	0.4
Nucleotides									

Adenosine	1.564	267.15	0.2	0.1	0.6			
Inosine	1.952	268.15	0.2	0.3	0.3			
Guanosine	2.488	283.15	0.1	0.2	0.4			
Xanthosine	2.822	284.1	0.2	0.4	0.4			
Guanosine diphosphate	0.713	443.0	0.1		0.6		-0.1	0.6
Cyclic GMP	0.932	345.0	0.0		0.3		-0.2	0.2
Adenosine monophosphate	4.673	347.1					0.1	0.6

H: the high-dose group; Pos: the positive group; MC: the model group; Con: the control group.