Cellular metabolites level assessment

Cells were seeded at a density of 2×10^6 per well in a 10 cm glass bottom cell culture dish and grown for 12 h in DMEM supplemented with 10% fetal bovine serum, 1% penicillin and streptomycin, cells were treated as indicated for 10 h. Cells were then trypsinized, washed and resuspended in methanol: acetonitrile: ddH_2O_2 (2:2:1, v/v) after indicated treatment. Then subjected to LC/MS analysis at LipidALL Technologies (Changzhou, China). Briefly, polar metabolites were extracted using 1000 µl of ice-cold methanol: H_2O (4:1, v/v), and incubated at 1500 rpm for 30 min at 4ºC. At the end of the incubation, samples were centrifuged for 10 min at 12000 rpm at 4ºC. Clean supernatant was transferred to a new tube. Extracts were dried in a SpeedVac under H_2O mode.

The dried extract was reconstituted in 2% acetonitrile in water prior to ultra-high performance liquid chromatography-high resolution mass spectrometry (UHPLC-HRMS) analysis on an Agilent 1290 II UPLC coupled to Sciex 5600 + quadrupole-TOF MS. LC-MS conditions were as previously described [1, 2]. For reverse phase chromatography, polar metabolites were separated on a Waters ACQUITY UPLC HSS T3 columns (1.7 µm, 2.1×100 mm, Dublin, Ireland), and mobile phase A was water containing 0.1% formic acid (v/v), and mobile phase B was acetonitrile. The column oven temperature was maintained at 40 °C, and autosampler was set at 10 °C. Injection volume was 10 µL. Flow rate was 0.35 mL/min. The following linear gradient was used: 0–1.0 min with 2% B, 1.0–6.0 min with 2%–42% B, 6.0-8.0 min with 42%–65% B, 8.0–10.0 min with 65%–76% B, 10.0-11.0 min with 76%–100% B, 11.0–14.0 min with 100%－100% B. While hydrophilic interaction liquid chromatography was conducted using an ACQUITY UPLC BEH Amide column (1.7 µm, 2.1 × 100 mm) (Waters, Dublin, Ireland). Mobile phase A was water containing 20 mM ammonium acetate in water: acetonitrile 90:10 (v/v) at pH9, and mobile phase B was 20 mM ammonium acetate in water: acetonitrile 10:90 (v/v) at pH9. The column oven temperature was maintained at 25 °C, and autosampler was set at 10 °C. Injection volume was 10 µL. Flow rate was 0.40 mL/min. The following linear gradient was used: 0–2.0 min with 95% B, 2.0–12.0 min with 95%-–50% B, 12.0-13.0 min with 50%–50% B, 13.0–13.1 min with 50%–95% B, 13.1-16.0 min with 95%–95%

B. MS parameters for detection were: ESI source voltage positive ion mode 5.5 kV, negative ion mode -4.5 kV; vaporizer temperature, 450°C; drying gas (N_2) pressure, 50 psi; nebulizer gas (N_2) pressure, 50 psi; curtain gas (N_2) pressure, 35 psi; The scan range was *m/z* 60-800. Information-dependent acquisition mode was used for MS/MS analyses of the metabolites. Collision energy was set at (±) 35 ± 15 eV. Data acquisition and processing were performed using Analyst® TF 1.7.1 Software (AB Sciex, Concord, ON, Canada). All detected ions were extracted using MarkerView 1.3 (AB Sciex, Concord, ON, Canada) into Excel in the format of two-dimensional matrix, including mass to charge ratio (m/z), retention time, and peak areas, and isotopic peaks were filtered. PeakView 2.2 (AB Sciex, Concord, ON, Canada) was applied to extract MS/MS data and perform comparisons with the Metabolites database (AB Sciex, Concord, ON, Canada), HMDB, METLIN, and standard references to annotate ion identities.

1. Tian H*, et al.* Precise Metabolomics Reveals a Diversity of Aging-Associated Metabolic Features. *Small Methods*, e2200130 (2022).

2. Xia JG*, et al.* Precise Metabolomics Defines Systemic Metabolic Dysregulation Distinct to Acute Myocardial Infarction Associated With Diabetes. *Arterioscler Thromb Vasc Biol* **43**, 581-596 (2023).