 Appendix S1.

Metabolome analysis

Animal tissue metabolite extraction for liquid chromatography (LC). A total of 30-50 mg frozen RV tissue was placed in a homogenization tube with zirconia beads (diameter, 5 and 3 mm). Next, 500 μl 1% formic acid/acetonitrile containing internal standards [cat. no. H3304-1002, Human Metabolome Technologies, Inc. (HMT)] was added to the tube, and the tissue was completely homogenized at 29.6 x g (4°C for 60 sec) using a bead shaker (Shake Master NEO; Bio Medical Science). Then, 167 μl ultrapure water was added and homogenization was performed for another 29.6 x g (4°C for 60 sec). The homogenate was then centrifuged at 2,300 x g at 4°C for 5 min. Subsequently, 600 μl supernatant was centrifugally filtered through a 3-kDa cutoff filter (Nanosep 3 K Omega; Pall Corporation) at 9,100 x g (4°C for 30 min) to remove macromolecules and further filtered using a Hybrid SPE-Phospholipid cartridge (30 mg/ml; Sigma-Aldrich; Merck KGaA) to remove phospholipids. The filtrate was evaporated to dryness under nitrogen and reconstituted in 100 μl 50% isopropanol (v/v) for metabolome analysis at HMT.

Animal tissue metabolite extraction for capillary electrophoresis (CE). A total of 20-40 mg frozen tissue was placed in a homogenization tube, along with zirconia beads (diameter, 5 and 3 mm). Next, 1,500 μl 50% acetonitrile/Milli-Q water containing internal standards (cat. no. H3304-1002; HMT) was added to the tube, after which the tissue was completely homogenized at 29.6 x g, 4°C, 60 sec) using a bead shaker. The homogenate was then centrifuged at 2,300 x g at 4°C for 5 min. Subsequently, 800 μl upper aqueous layer was centrifugally filtered through a Millipore 5 kDa cutoff filter (UltrafreeMC-PLHCC; HMT) at 9,100 x g at 4°C for 180 min to remove macromolecules. The filtrate was evaporated to dryness under nitrogen and reconstituted in 50 μl Milli-Q water for metabolome analysis at HMT.

Metabolome analysis (C-SCOPE). Metabolome analysis was performed using C-SCOPE (HMT) and CE time-of-flight mass spectrometry (TOFMS) for cation analysis and CE-tandem MS (CE-MS/MS) for anion analysis as previously described (1,2). Briefly, CE-TOFMS and CE-MS/MS analyses were performed using an Agilent CE capillary electrophoresis system with an Agilent 6210 time-of-flight mass spectrometer. The systems were controlled by Agilent G2201AA ChemStation software version B.03.01 (Agilent Technologies, Inc.) and Agilent 6460 Triple Quadrupole LC/MS, respectively. The systems were controlled by Agilent G2201AA ChemStation software version B.03.01 (Agilent Technologies, Inc.) for CE and connected by a fused silica capillary (inner diameter, 50 μm; length, 80 cm) to commercial electrophoresis buffer (cat. nos. H3301-1001 and H3302-1023 for cation and anion analyses, respectively; both HMT) as the electrolyte. TOFMS was scanned from m/z 50-1,000 (1) and a triple quadrupole mass spectrometer was used to detect compounds in dynamic Multiple Reaction Monitoring mode. Peaks were extracted using MasterHands automatic integration software ver.2.17.1.11 (Keio University, Tsuruoka, Yamagata, Japan) (3) and MassHunter Quantitative Analysis B.04.00 (Agilent Technologies, Inc.) was used to obtain peak information, including m/z, peak area and migration time (MT). Signal peaks were annotated according to the HMT metabolite database based on m/z value and MT. The peak area of each metabolite was normalized to internal standards and metabolite concentrations were evaluated using standard curves with three-point calibrations using each standard compound. Hierarchical cluster analysis (HCA) and principal component analysis (PCA) were performed as previously described (4) using MATLAB 7.14 (MathWorks) and R 2.15.1 program, respectively. Detected metabolites were plotted on metabolic pathway maps using ver.2.1.0 (Visualization and Analysis of Networks containing Experimental Data (VANTED) software (5).

Metabolome analysis (LC). Metabolome analysis was performed using LC package (HMT) and LC-TOFMS as previously described (1,2). Briefly, LC-TOFMS analysis was performed using an Agilent 1200 HPLC pump with an Agilent 6210 time-of-flight mass spectrometer. The systems were controlled by MassHunter B.06.00 (Agilent Technologies, Inc) and connected using an Octa Decyl Silyl column (inner diameter, 2 mm; length, 50 mm; particle size, 2 μm). The spectrometer was scanned from m/z 50-1,000, and peaks were extracted using MasterHands automatic integration software to obtain peak information including m/z, peak area, and retention time (RT) (3). Signal peaks corresponding to isotopomers, adductions and other products of known metabolites were excluded and the remaining peaks were annotated according to the HMT metabolite database based on m/z value and RT. The areas of the annotated peaks were normalized to internal standards and sample amounts to obtain relative levels of each metabolite. HCA and PCA (4) were performed using the proprietary MATLAB and R programs, respectively. Detected metabolites were plotted on metabolic pathway maps using VANTED software (5).

References

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3. Sugimoto M, Wong DT, Hirayama A, Soga T and Tomita M: Capillary electrophoresis mass spectrometry-based saliva metabolomics identified oral, breast and pancreatic cancer-specific profiles. Metabolomics 6: 78-95, 2010.
4. Yamamoto H, Fujimori T, Sato H, Ishikawa G, Kami K and Ohashi Y: Statistical hypothesis testing of factor loading in principal component analysis and its application to metabolite set enrichment analysis. BMC Bioinformatics 15: 51, 2014.
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Appendix S2.

Primers used for reverse transcription-quantitative (RT-q) PCR

The following rat primers were obtained from Qiagen GmbH: Peroxisome proliferator-activated receptor γ (cat. no. PPR47599A), carnitine palmitoyl transferase 1B (cat. no. PPR59395A), CD36 (cat. no. PPR43233A), mitofusin (Mfn) 1 (cat. no. PPR46301A), Mfn2 (cat. no. PPR44137A); optic atrophy 1 (cat. no. PPR44480A); mitochondrial transcription factor A (cat. no. PPR46611A), dynamin-related protein 1 (cat. no. PPR45077B) and β-actin (cat. no. PPR06570C).
Figure S1. PCA and heatmap of metabolome analysis. (A) PCA of metabolome data. PC1 and 2 represent 36.0 and 21.2% of total variation, respectively. Each dot denotes a single biological replicate (red, Su/Hx; yellow, Su/Hx + CH. (B) Heat map of each metabolite. The colors indicate relative expression (red, high; white, intermediate; green, low). PCA, principal components analysis; Su, SU5416; Hx, hypoxia; CH, chrysin.
Figure S2. Representative glycolysis and TCA cycle metabolites in the right heart of Su/Hx (red) and Su/Hx + CH (orange). TCA, tricarboxylic acid; Su, SU5416; Hx, hypoxia; CH, chrysin.