Macaques and growth conditions

Male *Macaca fascicularis* of about 18 years old were bought from Huazhen Biosciences (Guangzhou, China). All macaques were born from 16th August 2001 to 11th February 2004 (Supplemental Table S1) and housed in the animal rooms maintained at 16 ~ 26 °C and 40% ~ 70% room humidity on a 12-h/12-h light-dark cycle. All macaques of both NC and sDM groups were fed with the normal food (19.26 % protein, 5% fat, no sugar, and no cholesterol), whereas the HFHS monkeys were grown up on the same diet until switching to the high-fat and high-sugar (HFHS) food (≥ 30% sugar, ≥ 15% fat, ≥ 10.5% protein, and ≥ 0.5% cholesterol) on 1st March 2019 (Fig. 1a). After one year of food change, blood and urine samples were collected from all macaques after 12-14 hours of fasting for examination and the intravenous glucose tolerance test (IVGTT) was conducted. The routine hematological examination was performed on the Hematology Analyzer pocH-100iV (Sysmex, Kobe, Japan). Insulin was quantified using the Cobas E411 Analyzer (Roche, Basel, Switzerland), and other blood biochemical analyses, including the measurement of blood sugar, were done by the Cobas C311 Analyzer (Roche, Basel, Switzerland). Urinary analysis was done in the local hospital Guangzhou Conghua District Hospital of Traditional Chinese Medicine. IVGTT was done in the morning after 12-14 hours of fasting. In IVGTT, 50% (w/v) glucose solution was injected into the limb vein of the anesthetized monkeys (1ml/kg body weight) immediately after blood collection (0 min) from another limb. Then the blood samples were collected after 1, 3, 5, 10, 20, 40, and 60 min for both sugar and insulin analysis. All macaques were then sacrificed at about 18 years old, or 1.25 years after the switch of foods (Fig. 1a; Supplemental Table S1), and the liver, PB, and HPVB samples were collected. Both blood samples were centrifuged at 3,000 rpm for 10 min at 4°C immediately to separate sera. All samples were frozen in liquid nitrogen and stored at -80°C until use. The study protocol received prior approval from the Institutional Animal Care and Use Committee of Huazhen Biosciences.

Metabolite extraction

The hydrophilic and hydrophobic compounds were extracted using methanol/water and MTBE/methanol/water solvent systems, respectively. Samples were first thawed on ice. To extract hydrophilic metabolites from the tissue samples, 1 ml of methanol/water (7:3, v/v) was added to 50 mg of the liver, and homogenized with steel balls for 3 min at 30 Hz, followed by 1 min of a vortex. The homogenate was then centrifuged at 12,000 rpm for 10 min at 4 °C to collect the supernatant. Hydrophobic compounds were extracted from another 50 mg using a slightly modified protocol. Homogenization was done with 1 ml of MTBE/methanol (10:3, v/v) and 100 µl of water was mixed with the homogenate to extract before centrifugation. For the sera of PB and HPVB, 3 volumes (v/v) of methanol and a mixture of MTBE and methanol (10:3, v/v) were whirled with the serum samples for 3 min, followed by centrifugation at 12,000 rpm for 10 min at 4 °C. All collected supernatants were dried and store at -80°C until LC-MS/MS analysis. Internal standards were dissolved in the solvents before extraction.

LC-MRM-MS/MS analysis of metabolites

Both hydrophilic and hydrophobic extracts were analyzed using a UPLC-ESI-MS/MS system (UPLC, Shim-pack UFLC SHIMADZU CBM A system, SHIMADZU, Japan; MS, QTRAP® System, Sciex, Washington, USA). ACQUITY UPLC HSS T3 C18 column (1.8 µm, 2.1 mm*100 mm, Waters, Milford, MA, USA) was used for UPLC, working with the following parameters: the Column temperature of

40 °C, a flow rate of 0.4 ml/min, and injection volume of 2 μL. The analysis of hydrophilic metabolites used 0.1% formic acid (in water) and acetonitrile with 0.1% formic acid as mobile phases, with a gradient (V/V) as following: 95:5 at 0 min, 10:90 at 11.0 min, 10:90 at 12.0 min, 95:5 at 12.1 min, and 95:5 at 14.0 min. The two mobile phases for hydrophobic compounds were 0.04% acetic acid and acetonitrile with 0.04% acetic acid, and the gradient (V/V) program was 95:5 at 0 min, 5:95 at 11.0 min, 5:95 at 12.0 min, 95:5 at 12.1 min and 95:5 at 14.0 min. LIT and triple quadrupole (QQQ) scans were acquired in both positive and negative ion modes under the control of Analyst 1.6.3 software (Sciex, Washington, USA). The ion spray voltage (IS) of the ESI source was 5500 and -4500 V for positive and negative modes, respectively. Source temperature was set at 500 °C, ion source gas I (GSI), gas II (GSII), curtain gas (CUR) at 55, 60, and 25.0 psi, respectively, and collision gas (CAD) high. Instrument tuning and mass calibration in QQQ and LIT modes were performed with 10 and 100 μmol/L polypropylene glycol solutions, respectively. Specific sets of multiple reaction monitoring (MRM) transitions of various periods of retention time were monitored according to an in-house library of metabolites.

Raw data processing

Integration and correction of the peak areas corresponding to the targeted metabolites from the LC-MRM-MS/MS data were done with MulitQuant (version 3.0, Sciex, Washington, USA), followed by normalization against the total peak areas measured from each sample. The Automatic method of MultiQuent was used with the parameters specified as following: Gaussian smooth width: 0 points; RT half window: 30 s; min peak width: 2 points; min peak height: 800; noise percentage: 70.0 %; baseline sub window: 2 min; peak splitting: 2 points; RT tolerance: 0.2 min.