**LC-MS Lipidomics Methods for serum samples from mice under different drug treatments**

Serum samples from C57BLKsJ-db/db mice which were randomly grouped and treated with DG extract, KK01, metformin (positive control 1), rosiglitazone (positive control 2), and saline, separately, and the serum samples from C57BLKsJ mice (wild type) treated with saline, were provided from Wuhan Institute of Food and Medical Device Control, China. Samples were frozen in -80 oC and shipped to RTI-RCMRC on dry ice. Table 1 lists the sample ID and associated metadata provided.

For lipidomics analysis, the samples were first thawed on ice, and then mixed by vortexing for 4 min at 4,000 rpm, followed by centrifugation for 4 min at 4, 000 rcf to pellet. Pooled mouse serum from Sigma Aldrich (S7273) was used as QC samples. A 30 µL aliquot of each serum sample (including QC sample and experimental sample) was transferred to 2.0 mL Eppendorf Lo-Bind tubes and extracted with 600 µL of 2:1 dichloromethane: methanol (DCM) via vortex-mixing for 2 min at 4,000 rpm. Then, 120 µL of H2O was added and vortexed for 1 min at 4,000 rpm. Samples were incubated at room temperature for 10 min, then centrifuged at 16,000 rcf for 10 minutes at 10 °C. A 370 µL aliquot of the lower lipid-rich DCM layer was transferred to new labeled 2.0 mL Eppendorf Lo-Bind tubes and then lyophilized to complete dryness overnight. For immediate analysis, 300 µL of acetonitrile:isopropanol:H2O (65:30:5, v/v/v) was added to reconstitute the dried tissue extracts, and the samples were thoroughly mixed on a multi-tube vortexer for 10 min at 5000 rpm and centrifuged at 16,000 rcffor 4 min. The supernatants were transferred to labeled autosampler vials for data acquisition by LC-MS.

Broad spectrum lipidomics were conducted using a Waters Acquity UPLC coupled with Thermo Orbitrap Velos mass spectrometer. Lipids were separated on a Waters AcquityUPLC CSH C18 2.1 X 100 mm 1.7 µm operating at 50 °C using the reversed phase gradient separation with 10 mM ammonium formate in 60:40 H2O: acetonitrile containing 0.1% formic acid as mobile phase A and 10 mM ammonium formate in 90:10 isopropanol: acetonitrile containing 0.1% formic acid as mobile phase B. A 10 µL was injected into the instrument, and MS data was collected between 120-2000 m/z in both positive and negative modes.

**Table 1.** Mice serum Sample Information

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **DRCC ID** | **Sample matrix** | **Gene type** | **Phenotypic grouping and treatments** | **Preparation Type** |
| S\_56 | Serum | C57BLKsJ-db/db | Group1, DG extract high dose | Snap Frozen |
| S\_53 | Serum | C57BLKsJ-db/db | Group1, DG extract high dose | Snap Frozen |
| S\_7 | Serum | C57BLKsJ-db/db | Group1, DG extract high dose | Snap Frozen |
| S\_47 | Serum | C57BLKsJ-db/db | Group1, DG extract high dose | Snap Frozen |
| S\_19 | Serum | C57BLKsJ-db/db | Group1, DG extract high dose | Snap Frozen |
| S\_60 | Serum | C57BLKsJ-db/db | Group1, DG extract high dose | Snap Frozen |
| S\_67 | Serum | C57BLKsJ-db/db | Group1, DG extract high dose | Snap Frozen |
| S\_34 | Serum | C57BLKsJ-db/db | Group1, DG extract high dose | Snap Frozen |
| S\_28 | Serum | C57BLKsJ-db/db | Group1, DG extract high dose | Snap Frozen |
| S\_42 | Serum | C57BLKsJ-db/db | Group1, DG extract high dose | Snap Frozen |
| S\_25 | Serum | C57BLKsJ-db/db | Group 2, DG extract low dose | Snap Frozen |
| S\_4 | Serum | C57BLKsJ-db/db | Group 2, DG extract low dose | Snap Frozen |
| S\_54 | Serum | C57BLKsJ-db/db | Group 2, DG extract low dose | Snap Frozen |
| S\_16 | Serum | C57BLKsJ-db/db | Group 2, DG extract low dose | Snap Frozen |
| S\_73 | Serum | C57BLKsJ-db/db | Group 2, DG extract low dose | Snap Frozen |
| S\_77 | Serum | C57BLKsJ-db/db | Group 2, DG extract low dose | Snap Frozen |
| S\_38 | Serum | C57BLKsJ-db/db | Group 2, DG extract low dose | Snap Frozen |
| S\_3 | Serum | C57BLKsJ-db/db | Group 2, DG extract low dose | Snap Frozen |
| S\_36 | Serum | C57BLKsJ-db/db | Group 2, DG extract low dose | Snap Frozen |
| S\_57 | Serum | C57BLKsJ-db/db | Group 2, DG extract low dose | Snap Frozen |
| S\_15 | Serum | C57BLKsJ-db/db | Group 3, KK 01 high dose | Snap Frozen |
| S\_61 | Serum | C57BLKsJ-db/db | Group 3, KK 01 high dose | Snap Frozen |
| S\_20 | Serum | C57BLKsJ-db/db | Group 3, KK 01 high dose | Snap Frozen |
| S\_59 | Serum | C57BLKsJ-db/db | Group 3, KK 01 high dose | Snap Frozen |
| S\_66 | Serum | C57BLKsJ-db/db | Group 3, KK 01 high dose | Snap Frozen |
| S\_48 | Serum | C57BLKsJ-db/db | Group 3, KK 01 high dose | Snap Frozen |
| S\_5 | Serum | C57BLKsJ-db/db | Group 3, KK 01 high dose | Snap Frozen |
| S\_33 | Serum | C57BLKsJ-db/db | Group 3, KK 01 high dose | Snap Frozen |
| S\_76 | Serum | C57BLKsJ-db/db | Group 3, KK 01 high dose | Snap Frozen |
| S\_51 | Serum | C57BLKsJ-db/db | Group 3, KK 01 high dose | Snap Frozen |
| S\_29 | Serum | C57BLKsJ-db/db | Group 4, KK 01 low dose (i.p.) | Snap Frozen |
| S\_43 | Serum | C57BLKsJ-db/db | Group 4, KK 01 low dose (i.p.) | Snap Frozen |
| S\_39 | Serum | C57BLKsJ-db/db | Group 4, KK 01 low dose (i.p.) | Snap Frozen |
| S\_12 | Serum | C57BLKsJ-db/db | Group 4, KK 01 low dose (i.p.) | Snap Frozen |
| S\_50 | Serum | C57BLKsJ-db/db | Group 4, KK 01 low dose (i.p.) | Snap Frozen |
| S\_37 | Serum | C57BLKsJ-db/db | Group 4, KK 01 low dose (i.p.) | Snap Frozen |
| S\_14 | Serum | C57BLKsJ-db/db | Group 4, KK 01 low dose (i.p.) | Snap Frozen |
| S\_79 | Serum | C57BLKsJ-db/db | Group 4, KK 01 low dose (i.p.) | Snap Frozen |
| S\_82 | Serum | C57BLKsJ-db/db | Group 4, KK 01 low dose (i.p.) | Snap Frozen |
| S\_23 | Serum | C57BLKsJ-db/db | Group 4, KK 01 low dose (i.p.) | Snap Frozen |
| S\_74 | Serum | C57BLKsJ-db/db | Group 5, metformin | Snap Frozen |
| S\_58 | Serum | C57BLKsJ-db/db | Group 5, metformin | Snap Frozen |
| S\_1 | Serum | C57BLKsJ-db/db | Group 5, metformin | Snap Frozen |
| S\_8 | Serum | C57BLKsJ-db/db | Group 5, metformin | Snap Frozen |
| S\_49 | Serum | C57BLKsJ-db/db | Group 5, metformin | Snap Frozen |
| S\_13 | Serum | C57BLKsJ-db/db | Group 5, metformin | Snap Frozen |
| S\_75 | Serum | C57BLKsJ-db/db | Group 5, metformin | Snap Frozen |
| S\_44 | Serum | C57BLKsJ-db/db | Group 5, metformin | Snap Frozen |
| S\_71 | Serum | C57BLKsJ-db/db | Group 5, metformin | Snap Frozen |
| S\_52 | Serum | C57BLKsJ-db/db | Group 6, rosiglitazone | Snap Frozen |
| S\_21 | Serum | C57BLKsJ-db/db | Group 6, rosiglitazone | Snap Frozen |
| S\_65 | Serum | C57BLKsJ-db/db | Group 6, rosiglitazone | Snap Frozen |
| S\_32 | Serum | C57BLKsJ-db/db | Group 6, rosiglitazone | Snap Frozen |
| S\_72 | Serum | C57BLKsJ-db/db | Group 6, rosiglitazone | Snap Frozen |
| S\_69 | Serum | C57BLKsJ-db/db | Group 6, rosiglitazone | Snap Frozen |
| S\_2 | Serum | C57BLKsJ-db/db | Group 6, rosiglitazone | Snap Frozen |
| S\_45 | Serum | C57BLKsJ-db/db | Group 6, rosiglitazone | Snap Frozen |
| S\_46 | Serum | C57BLKsJ-db/db | Group 6, rosiglitazone | Snap Frozen |
| S\_18 | Serum | C57BLKsJ-db/db | Group 6, rosiglitazone | Snap Frozen |
| S\_62 | Serum | C57BLKsJ-db/db | Group 7, saline | Snap Frozen |
| S\_24 | Serum | C57BLKsJ-db/db | Group 7, saline | Snap Frozen |
| S\_40 | Serum | C57BLKsJ-db/db | Group 7, saline | Snap Frozen |
| S\_27 | Serum | C57BLKsJ-db/db | Group 7, saline | Snap Frozen |
| S\_64 | Serum | C57BLKsJ-db/db | Group 7, saline | Snap Frozen |
| S\_41 | Serum | C57BLKsJ-db/db | Group 7, saline | Snap Frozen |
| S\_31 | Serum | C57BLKsJ-db/db | Group 7, saline | Snap Frozen |
| S\_68 | Serum | C57BLKsJ-db/db | Group 7, saline | Snap Frozen |
| S\_70 | Serum | C57BLKsJ-db/db | Group 7, saline | Snap Frozen |
| S\_22 | Serum | C57BLKsJ-db/db | Group 7, saline | Snap Frozen |
| S\_17 | Serum | C57BLKsJ (WT) | Group 8, saline | Snap Frozen |
| S\_78 | Serum | C57BLKsJ (WT) | Group 8, saline | Snap Frozen |
| S\_9 | Serum | C57BLKsJ (WT) | Group 8, saline | Snap Frozen |
| S\_55 | Serum | C57BLKsJ (WT) | Group 8, saline | Snap Frozen |
| S\_11 | Serum | C57BLKsJ (WT) | Group 8, saline | Snap Frozen |
| S\_30 | Serum | C57BLKsJ (WT) | Group 8, saline | Snap Frozen |
| S\_6 | Serum | C57BLKsJ (WT) | Group 8, saline | Snap Frozen |
| S\_80 | Serum | C57BLKsJ (WT) | Group 8, saline | Snap Frozen |
| S\_63 | Serum | C57BLKsJ (WT) | Group 8, saline | Snap Frozen |
| S\_26 | Serum | C57BLKsJ (WT) | Group 8, saline | Snap Frozen |
| S\_35 | Serum | C57BLKsJ-db/db | Group 8, saline | Snap Frozen |
| S\_81 | Serum | C57BLKsJ-db/db | Group 9, saline (i.p.) | Snap Frozen |
| S\_10 | Serum | C57BLKsJ-db/db | Group 9, saline (i.p.) | Snap Frozen |
| S\_83 | Serum | C57BLKsJ-db/db | Group 9, saline (i.p.) | Snap Frozen |
| P\_1 | Serum | Pool serum from Sigma | External Pool | Frozen |
| P\_2 | Serum | Pool serum from Sigma | External Pool | Frozen |
| P\_3 | Serum | Pool serum from Sigma | External Pool | Frozen |
| P\_4 | Serum | Pool serum from Sigma | External Pool | Frozen |
| P\_5 | Serum | Pool serum from Sigma | External Pool | Frozen |
| P\_6 | Serum | Pool serum from Sigma | External Pool | Frozen |
| P\_7 | Serum | Pool serum from Sigma | External Pool | Frozen |
| P\_8 | Serum | Pool serum from Sigma | External Pool | Frozen |
| P\_9 | Serum | Pool serum from Sigma | External Pool | Frozen |
| P\_10 | Serum | Pool serum from Sigma | External Pool | Frozen |
| P\_11 | Serum | Pool serum from Sigma | External Pool | Frozen |

Note: i.p., intraperitoneal injection. Without specific indication, drugs or saline were administrated with intragastric gavage.