GC-MS analysis of tricarboxylic acid cycle (TCA) metabolites

Targeted profiling of TCA metabolites was conducted at the Mayo Clinic by gas chromatography mass spectrometry (GC-MS) as previously described[55,56], with a few modifications. Briefly, 5 mg of tissue were homogenized in 1X PBS on an Omni bead ruptor (Omni International, Kennesaw, GA) prior to adding 20 µl of internal solution containing U-^{13}C labeled analytes ($^{13}C_3$ sodium lactate, $^{13}C_4$ succinic acid, $^{13}C_4$fumaric acid, $^{13}C_4$ alpha ketoglutaric acid, $^{13}C_4$ malic acid, $^{13}C_4$ aspartic acid, $^{13}C_5$ 2-hydroxyglutaric acid, $^{13}C_5$glutamic acid, $^{13}C_6$ citric acid, $^{13}C_2$,^{15}N glycine, $^{13}C_2$ sodium pyruvate). For plasma, 50 µl were used. The proteins were removed by adding 300 µl of chilled methanol and acetonitrile solution to the sample mixture. After drying the supernatant in the speedvac, the sample was derivatized with ethoxime and then with MtBSTFA + 1% tBDMCS before it was analyzed on an Agilent 5977B GC/MS (Santa Clara, California) under single ion monitoring conditions using electron ionization. Concentrations of lactic acid (*m/z* 261.2), fumaric acid (*m/z* 287.1), succinic acid (*m/z* 289.1), ketoglutaric acid (*m/z*360.2), malic acid (*m/z* 419.3), aspartic acid (*m/z* 418.2), 2-hydroxyglutaratic acid (*m/z* 433.2), cis aconitic acid (*m/z*459.3), citric acid (*m/z* 591.4), and isocitric acid (*m/z* 591.4), glutamic acid (*m/z* 432.4) were measured against 7-point calibration curves that underwent the same derivatization procedure using ethoxyamine and MtBSTFA. The injection volume was 1uL, and the flow rate was 1mL/min. Table 8 shows the temperature program. Agilent MassHunter was used for data processing.

Table 8. GC temperature gradient program

	Rate (°C/min)	Value (°C)	Hold Time (min)	Run Time (min)
Initial		120	0.5	0.5
Ramp 1	25	188	0	3.22
Ramp 2	7	275	0	15.649
Ramp 3	35	320	1.066	18