LC-MS/MS analysis of branched-chain keto acids

Targeted profiling of branched-chain keto acid metabolites was conducted at Duke University. Ten µl of plasma containing isotopically labeled ketoleucine (KIC)-d3, ketoisovalerate (KIV)-$^{13}C_5$ (Cambridge Isotope Laboratories), and 3-methyl-2-oxovalerate (KMV)-d8 (Toronto Research Chemicals, Canada) internal standards were deproteinated with 150 µl of 3M perchloric acid. Two hundred µl of tissue homogenate prepared at 100 mg/ml in 3M perchloric acid were centrifuged at 14,000 x g for 5 minutes. Two hundred µl of 25 M o-phenylenediamine (OPD) in 3M HCl were added to the plasma and tissue supernatants, and the samples were incubated at 80°C for 20 minutes. Keto acids were extracted with ethyl acetate as previously described[45,46]. The extracts were dried under nitrogen, reconstituted in 200 mM ammonium acetate, and analyzed on a Xevo TQ-S triple quadrupole mass spectrometer coupled to an Acquity UPLC (Waters) controlled by the MassLynx 4.1 operating system. The analytical column (Waters Acquity UPLC BEH C18 Column, 1.7 µm, 2.1 × 50 mm) was used at 30°C. 10 µl of the sample were injected onto the column and eluted at a flow rate of 0.4 ml/min. The gradient consisted of 45% mobile phase A (5 mM ammonium acetate in water) and 55% mobile phase B (methanol) for 2 minutes, followed by a linear gradient to 95% B from 2 to 2.5 minutes, held at 95% B for 0.7 minutes, returned to 45% A, and finally the column was re-equilibrated at initial conditions for 1 minute. The total run time was 4.7 minutes. Mass transitions of *m/z* 203 → 161 (KIC), 206 → 161 (KIC-d3), 189 → 174 (KIV), 194 → 178 (KIV-$^{5}C_{13}$), 203 → 174 (KMV), and 211 → 177 (KMV-d8) were monitored in positive ion mode. The endogenous keto acids were quantified using calibrators prepared by spiking dialyzed fetal bovine serum with authentic keto acids (Sigma-Aldrich).