Flow injection MS/MS analysis of acyl CoAs

Targeted profiling of acyl CoAs was conducted at Duke University. 500 µl of tissue homogenate prepared at 50 mg/ml in isopropanol/0.1 M KH_2PO_4 (1:1) was extracted with an equal volume of acetonitrile and centrifuged at 14,000 x g for 10 min as previously described[47,48]. The supernatants were acidified with 0.25 ml of glacial acetic acid, and acyl CoAs were further purified by solid phase extraction (SPE) using 2-(2-pyridyl) ethyl functionalized silica gel (Sigma-Aldrich) as described[49]. The SPE columns were conditioned with 1 ml of acetonitrile/isopropanol/water/glacial acetic acid (9/3/4/4 : v/v/v/v). Following application and flow through of the supernatant, the SPE columns were washed with 2 ml of acetonitrile/ isopropanol/water/glacial acetic acid (9/3/4/4 : v/v/v/v). Acyl CoAs were then eluted with 2 ml of methanol/250 mM ammonium formate (4/1 : v/v) and analyzed by flow injection MS/MS analysis using positive ion mode on a Xevo TQ-S, triple quadrupole mass spectrometer (Waters), employing methanol/water (80/20, v/v) containing 30 mM ammonium hydroxide as the mobile phase[50]. Spectra were acquired in the multichannel acquisition mode monitoring the neutral loss of 507 amu (phosphoadenosine diphosphate) and scanning from *m/z* 750 to 1100. Heptadecanoyl CoA was employed as an internal standard. The endogenous Acyl CoAs were quantified using calibrators prepared by spiking tissue homogenates with authentic Acyl CoAs (Sigma-Aldrich) having saturated acyl chain lengths C0 - C18. Corrections for the heavy isotope effects, mainly ^{13}C, to the adjacent m+2 spectral peaks in a particular chain length cluster were made empirically by referring to the observed spectra for the analytical standards.