Amino Acids and TCA cycle byproducts

Fifty pairs of ovaries from each genotype were dissected in cold 1xPBS. Since nosGAL4>ACC RNAi ovaries lacked vitellogenic egg chambers, the vitellogenic egg chambers from control ovaries were removed using forceps, leaving the transparent regions for metabolite extraction. Metabolites from each sample (containing about 12.5 µg DNA) were extracted using 200 µl metabolite extraction solvent (2:2:1 acetonitrile: methanol: ddH2O) and stored at -20°C overnight. Samples were homogenized by vortexing for 5 sec followed by cold bath sonication for 5 min, repeated twice. Tissue debris was pelleted by centrifugation at 15871x g (rcf) for 10 min at 4°C. A total of 200 µl metabolite- containing supernatant was transferred into a new tube. The supernatant was freeze-dried for at least 3 h using a freeze drier (VirTis BenchTop K). Dried samples were kept at -80°C until analyzed for amino acids and TCA cycle byproducts.

The samples were derivatized by bis(trimethylsilyl)- trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS) and analyzed using Agilent 7890B gas chromatography coupled with 7250 quadrupole time-of-flight mass spectrometer (GC-Q-TOF/MS) equipped with electron ionization (EI). The separation was performed on Zorbax DB5- MS+10 m Duragard Capillary Column (30 m x 0.25 mm x 0.25 mm, Agilent). The GC temperature profile was held at 60℃ for 1 minutes and then raised at 10℃/min to 325℃ and held at 325℃ for 10 minutes. The transfer line and the ion source temperature were set at 300 ºC and 280 ºC, respectively. The mass-range monitored was from 50 to 600 Daltons.The data acquisition and analysis were performed on MassHunter Workstation software. Mass spectra were compared against the NIST 2017, Fiehn and Wiley Registry 11th Edition mass spectral library. The Agilent MassHunter Unknows Analysis software was used for deconvolution of the signals. The results were imported into the Agilent Mass Profiler Professional software (Agilent Technologies,15.1, Santa Clara, CA, USA) for further peak alignment.