**Summary of the study**

Reproduction is closely tied to nutrient intake and lipid metabolism, with imbalances often leads to reproductive failure. We characterized the metabolic mechanisms mediated by Acetyl-CoA Carboxylase (ACC, a rate-limiting enzyme for fatty acid synthesis) that support oogenesis and discovered that ACC regulates nutrient-responsive TOR signaling to maintain endosomal trafficking, crucial for oocyte determination. ACC deficiency shifts metabolism toward fatty acid oxidation (FAO), fueling the TCA cycle and electron transport chain (ETC), which hyperactivates TOR signaling. This results in excessive protein synthesis, disrupting endosomal trafficking and impairing germ cell differentiation. Restoring balance through FAO or TOR inhibition, reducing protein synthesis, or adjusting dietary protein intake corrects these defects. Our findings reveal a critical link between lipid metabolism and nutrient-sensing pathways in oogenesis, offering potential therapeutic strategies for metabolic disorders affecting reproduction.

Acetyl-CoA extraction and detection protocol

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 Ultra-Performance Liquid Chromatography system (ACQUITY UPLC, Waters, Millford, MA) was coupled online to the Waters Xevo TQ-S triple quadrupole mass spectrometer (Waters, Milford, USA). The sample was separated with ACQUITY BEH Amide column (1.7μm particle size, 2.1 × 100 mm, Waters). The UPLC was operated at a flow rate of 0.3 mL/min and column temperature of 40℃. The composition of mobile phase A was water containing 20mM ammonium acetate and 0.3% ammonium hydroxide, mobile phase B was 90% acetonitrile containing containing 20mM ammonium acetate and 0.3% ammonium hydroxide. Characteristic MS transitions were monitored using positive multiple reaction monitoring (MRM) mode for acetyl-CoA (m/z, 810>303). Data acquisition and processing were performed using MassLynx version 4.1 and TargetLynx software (Waters Corp.).