Don’t forget to calibrate!!!

12 week rats and 30 week rats should be separate batches, separate QCs, separate everything

QC pull 50-100 uL from all samples (keep age batches separate)

Include Splash (not ultimate splash) and 13C Amino acids (AAs are in -20 next to Katie’s bench in other room)

* The 2 standard mixes get mixed into the n-butanol at 5 uL/mL final concentration in the n-butanol extraction solvent
* This avoids having to pipette standard mix into each of your sample tubes 60x

QC dilution series. (dry down 200 uL, 100, 50, 25). Might only need to do replicates for one of the batches

Check that we have enough HILIC solvent

HILIC on Brutus, RP on Focus

**Methods**

Plasma sample prep

1. Make 35 mL of extraction solvent (3:1:1 n-butanol:acetonitrile:water v/v/v) (21 mL n-butanol, 7 mL each ACN and water)
   1. Add 5 uL/mL (175 uL) Splash standard mix (not UltimateSplash)
   2. Add 5 uL/mL (175 uL) of 1:100 diluted Amino Acids mix.
2. Thaw plasma samples on ice
3. Measure 5 uL plasma into extraction vial
4. Add 500 uL extraction solvent
5. Vortex 10 sec
6. Let all samples sit on ice at least 10 minutes
7. Centrifuge at 14,000 g for 2 mins at 4 deg C to precipitate protein.
8. For each plasma sample, extract 100 uL plasma each into two vials, one for RP, one for HILIC.
   1. Make extraction blank vial with no plasma
   2. Make resuspension solvent blank
   3. Make a pooled QC sample with 100 uL from all samples
   4. For pooled QC, do dilution series in vials with 200, 150, 100, 50, 25 of plasma.
   5. Make 3 vials of 100 uL QC (to ensure enough sample for multiple injections of QC during runs)
9. Dry down on speedvac
10. Resuspend in solvent for LCMS analysis (vortex 10s)
    1. Lipidomics: 50 uL 9:1 methanol:toluene v/v
    2. HILIC metabolomics: 25 uL 1:1 acetonitrile:water v/v

Food sample prep

1. Thaw samples on ice
2. Pulverize in pestle and mortar for 2 minutes
3. Measure 33 mg sample and place in 1.5 mL eppendorf with 1 mL extraction solvent (6:2:2 n-butanol:acetonitrile:water v/v/v)
4. Make an extraction blank as well, so 1.5 mL Eppendorf with 1 mL extraction solvent and no food
5. Place tube in cold sonicator at 14 degC, 20 seconds on, 10 seconds off, for 20 minutes total.
6. Centrifuge tubes at 14,000 g for 2 mins at 4 deg C.
7. Remove 200, 150, 100, 50 uL into amber autosampler vials with glass insert.
   1. Do 2 of each (1 for lipids, 1 for polar metabolites)
   2. For extraction blank, do 100 uL
8. Dry down on speedvac
9. Resuspend in solvent for LCMS analysis
   1. Lipidomics: 50 uL 9:1 methanol:toluene v/v
   2. HILIC metabolomics: 25 uL 1:1 acetonitrile:water v/v