Basic Information for Directing a Mouse Atherosclerosis Project

For an excellent review of the fundamentals of designing and analyzing mouse atherosclerosis projects, refer to Daugherty & Rateri (2005), *Methods* 36:129-138.

1. It is better to set up simultaneous *multiple* breeding cages that will give rise to many, closely coordinated waves of mice for the final study rather than to do it in piecemeal fashion. Make sure the mice are ear-marked at the time of genotyping.

2. When the pups for the study are weaned, place in cages of same sex. Each cage should have a similar number of mice. Do not enter into the study any mice that appear abnormal.

3. Keep a careful table that identifies the (1) breeding cage/parents from which the mouse arose; (2) genotype; (3) sex; (4) birth date; (5) two weights: at 6 weeks of age (which the time special diet usually begins) and at time of harvest. Assign each mouse an ID# whose code is only known by the director of the project—George must be blinded as to the identity of the mice.

4. On a dedicated calendar, note dates in future for start of special diet feeding (*e.g.*, Western diet if applicable) and dates of harvest. Make sure proper diets are received well before diet-start date. Inform George and Ira one week before harvest date.

5. Make notations on this table of any unusual events, such as empty food cages, power shutdowns, and health issues related to the mouse room (*e.g.*, pinworm outbreak) or to individual mice (*e.g.*, fighting with cage mates, loss of hair, bleeding, etc.).
6. On late afternoon prior to harvest day: place mice in fresh, clean cage and fast overnight.

7. On morning of harvest, work with George to obtain tail clips, weights, and blood for plasma cholesterol and lipoproteins. Label three sets of Eppendorf tubes with the ID#'s of the mice:

    1. The first set is for the blood and should be pre-filled with 20 µl of 100mM mM EDTA in PBS. These tubes are centrifuged at 14,000 rpm for 10 min in the cold room to isolate plasma.
    2. Add this plasma to the second set of tubes, which should be pre-filled with 10 µl sodium azide from a 2% stock solution; store the plasma at 4°C.
    3. The third set of tubes is for the tail clips (in case genotype verification is needed)—store these at -80°C.

8. George will then harvest proximal aorta and brachiocephalic artery and process by freezing in OCT or by paraffin embedding, depending on the procedure. Make sure that you and George are in sync regarding the identification of the mice and specimens.

9. From the fresh plasma, prepare HDL by precipitation of apoB-containing lipoproteins (via kit), and store the HDL under argon at 4°C. Within one month, assay cholesterol and, if necessary, triglyceride in the plasma (total cholesterol [TC]) and HDL using the colorimetric kit. Note that the cholesterol assay is best in the 2-9 mg/ml range, although HDL is usually below this. If cholesterol above 12-15 mg/ml, you will need to dilute with 0.15 M NaCl/1 mM EDTA.

10. For FPLC lipoprotein profile: assay a small aliquot of *fresh* plasma samples for total cholesterol and HDL cholesterol. After retrieving the data, find mice whose TC and HDL levels are close to that of the mean for that sex and genotype based upon your previous data. For each sex and genotype, pool the remaining plasma from 2-3 mice that meet these criteria and fractionate by FPLC. Assay 80 µl of each fraction for cholesterol.

    1. A minimum of 300 µl pooled plasma is needed. Dilute with FPLC buffer (below) if cholesterol is > 15 mg/ml.
    2. We use Henry Ginsberg's FPLC, run by Colleen on P&S 9 th floor cold room.

3. Buffer (0.15 M NaCl/1 mM EDTA/0.02% sodium azide) must be filtered to remove particles using our sterilizing 1-L filters, degassed in an Erlenmeyer flask under vacuum, and pre-cooled. Use Program #2 on FPLC—flow rate is 0.3 ml/min, and fractions are ~0.5 ml