Aim 1: **Identify the physiological mechanisms contributing to energy conservation and insulin resistance during pregnancy.** Age matched pregnant and non-pregnant female mice will be compared in this study. Energy expenditure, insulin sensitivity, and macronutrient absorptive efficiency will be evaluated

Background:

Methods:

*Animal care and use*

Nine week old virgin C57Black 6/J mice were ordered from Jackson Laboratory (n=21, 14 female, 7 male). Animals were held in a 12:12 light dark cycle, temperature and humidity-controlled facility. Animals were allowed to acclimate to the environment for two weeks. At 11 weeks of age, females were singly housed and given *ad libitum* access to normal chow (5% fat, 24% protein, 3.7% sucrose, 32% starch). Females were randomized to 2 groups; distilled drinking water and no mating (n=7), distilled drinking water and mating (n=7). Food intake (grams per week) and water intake (mLs per week) was recorded each week. Animals were acclimatized to this diet treatment for one week prior to mating. All protocols (name the protocol) were approved by the university of Michigan IACUC office.

*Mating*

At mating, males were added to females’ existing cages in monogamous pairs (n=7). Dams were examined for copulatory plugs each day until plug was evident. This was considered gestation day 1. Males were removed from cages on gestation day 19 to prevent a second pregnancy after delivery.

*Statistics*

All statistical analyses were conducted in R, version ().Repeated measures, such as body composition, food and water intake, and insulin tolerance testing were modeled using mixed effects linear modeling using the lme4 package. To test for statistical significance between mixed linear models, anovas were used. Fasting blood glucose was modeled using two way anova. Insulin tolerance was modeling using normalized values to baseline for blood glucose and mixed linear effects modeling was used for both normalized and area under the curve (AUC) values.

*Body Composition:*

Once a week, Dams weight was measured weekly using a electronic scale (). Body composition including fat mass, lean mass, and free water was assessed indirectly via EchoMRI ().

*Insulin Sensitivity:*

*Insulin tolerance testing*

On gestational day 16 (based on appearance of copulatory plug representing gestation day 1), dams were fasted for 6 hours with *ad libitum* access to either water, or dexamethasone in drinking water. After 6 hour fast, blood glucose was taken using a glucometer and tail clip. Females were given insulin injections (0.75 units/kg body weight; Humulin U100 in cold sterile, filtered Phosphate buffered saline (PBS)) and blood glucose was tested using a glucometer at 15 minute intervals for 2 hours.

* Dissection and culture of pancreas around G16-18? We could also look at GSIS in vivo.

*Blood Collection:*

24 hours after the insulin tolerance test was conducted eye bleeds were conducted on pregnant dams at 2 time points (ZT12 and then ZT0). Bleeds were done retro-orbitally under light iso-fluorane anesthesia.

* Cort
* Insulin

*Energy Expenditure:*

* *CLAMS – see wheel running paper*
* *Feeding Efficiency – look at thesis for this reference*

*Digestive Physiology:*

* *Bomb calorimetry of fecal matter*
  + *Start with energy*
  + *Progress to macronutrients if energy abs different*
* *Dissection of the SI – perhaps histological examination*

Expected Results and Potential Pitfalls: