**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.**

Subaim 1.1: Insulin sensitivity

Subaim 1.2: Energy Expenditure

Subaim 1.3: Absorptive efficiency

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 not mated (n=7), distilled drinking water and mated(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 (protocol number ).

*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.

*Body Composition:*

Once a week, Dams weight was measured weekly using an electronic scale (). Body composition including fat mass, lean mass, and free water was assessed indirectly via magnetic resonance imaging (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. If animals began to exhibit moribund behaviors, 300 units of 10% glucose in cold sterile filtered PBS was administered and subsequent BG measurements were omitted from the ITT.

* Dissection and culture of pancreas around G16-18? We could also look at GSIS in vivo.
* Check the liver and fat for insulin signaling stuff (be sure everyone has been fasting the same amount of time for this to be usable.)

*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. Samples were allowed to clot on ice. Then samples were spun down to separate serum from cellular components of blood in a cooled (4 degrees C) microcentrifuge (5000 rpm for 20 minutes). Serum was pipetted off by hand and stored at -80 degrees C until samples were analyzed.

*Blood Glucose*

Continuous glucose monitoring will be employed to capture the full diurnal pattern of glycemic health during the course of pregnancy.

*Hormone analyses:*

To determine concentration of hormone in maternal blood samples, ELISA was utilized on serum collected at ZT0 and ZT12. Corticosterone ELISA(Crystal Chem catalog number: 90080) was followed here, and analysis of content was based on \_\_\_\_. Serum Insulin concentration will also be evaluated in this manner.

*Energy Expenditure:*

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

Animals will be singly housed in a metabolic phenotyping cage after mating and discovery of a

*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, ANOVA 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.

*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:

Although pregnancy has been noted to induce insulin resistance and there is a general trend toward energy conservation, there are many things that are incompletely testing in pregnant mice. Specifically,

* May not increase overall energy expenditure, but would increase EE in response to post prandial period (TEF)(Peterson/Ravussin, Obesity 2019)
* Potential pitfall, may only be helpful in mice that are obese/metabolically unhealthy at baseline.
* Use of the CLAMs may result in greater loss of implanted pups due to stress
  + Will expose them prior to mating and put in after plug has been confirmed.
* If there is no difference is the bomb calorimetry of the total kcal amount in stool, then there should be a macronutrient-specific analysis completed. There is evidence that nutrient absorption is altered in pregnancy (micronutrients), but this hasn’t been done for macronutrients.