1. **OUTLINE:**Figure 1 - experimental design
   * Mom feeding graphic
   * Offspring diet and experimental rundown graphic
2. FIgure 2 - early life food intake and glycemic control/ pre HFD
   * Body mass/comp up to PND 70 (when HFD starts)
   * Food intake of NCD,  - leaning towards cumulative intake averaged by cage then group. Should I report weekly non-cumulative?
   * GTT pre HFD, ITT pre HFD (both AUC and time-course images)
   * Fasting glucose
3. Figure 4 - Body composition and food intake responses to Post HFD feeding
   * A-c Body mass/comp PND 70 to sacrifice
   * D (e)  Food intake with HFD - again leaning toward cumulative  intake
   * F.g.h.i HFD GTT and ITT (AUC and time-course images)
   * H.i Insulin values from GSIS (in vivo)
   * Figure 5 might need to be glycemic control only (too crowded)
4. Figure 6 Molecular - still need to look at this - discussion
   * In Vitro GSIS
   * Want to look at pancreas tissue - need to go grab whichever ones aren’t used for Brigid’s experiment
     1. Should we get pancreas for histology and look at markers for calcium channels or  actual insulin in the tissues, pdx-1 transcripts?

Look at:

1. Blood FA/TG/lipids to go along with glucose (3/4d depending on timing of bleed)
2. Islet vs endocrine - GIP/GLP/ etc from endocrine effect
3. Direct reading to IUGR more (mechanisms of sex-specific HFD induced IUGR)

**Abstract   
Introduction  
Methods**

*Animal care and use*

Virgin female C57/bl6J mice were obtained from Jackson Laboratory at XX days of age. All animals were maintained on a 12-hour (12 dark:12 light) dark cycle in a temperature and humidity controlled room. After one week of acclimatization, they were single housed and dietary treatment began (either eTRF or AL feeding). After one week of dietary treatment, age-matched males were introduced into cages for breeding. Males were kept in the female cage until copulatory plug appeared. The day a copulatory plug appeared was designated gestational day 0.5 (E 0.5). After birth (post-natal day, PND 0.5), offspring were weighed multiple times (PND 3.5, 7.5, 14.15) before reaching weaning age (PND 21.5). Litters were culled to 4 pups (2 males, 2 females, when possible) at PND 3.5. At weaning,

*Maternal dietary treatment*

Dams were randomized to either early time-restricted feeding (eTRF) or *ad libitum* (AL) feeding during gestation (n= eTRF, n=AL). Dams fed AL had 24 hour access to NCD (Lab diet, 5L0D) and water. Dams fed eTRF had 6 hours of food access over the early portion of the dark cycle (zeitgeber time , ZT 14-ZT 20) for the whole of pregnancy. All animals were transferred to a clean cage at the ZT20, allowing for similar levels of handling of all experimental animals. After birth, all dams were switched to AL feeding.

*Offspring growth and monitoring*

Pups born to either eTRF or AL dams were weighed within 24 hours of birth. Litters were reduced to 4 pups (2 male, 2 female when possible) at PND 3.5. Pups were weighed again at PND 7.5, 14.5, and 21.5. At PND 21.5, offspring were weighed, and body composiotion was assessed using EchoMRI before being weaned by sex and maternal feeding regimen and housed 4-5 per cage.

*Insulin Tolerance and Glucose Tolerance Testing*

Baseline glucose and insulin tolerance were assessed at young adulthood during NCD diet period (~ postnatal day 70). Animals were transferred into a cage with no food during the early light cycle, with water freely available. After 6 hours, fasting blood glucose was assessed using tail clip and a handheld glucometer (OneTouch Ultra). Shortly thereafter, an intraperitoneal injection of insulin (Humulin, u-100; 0.75u/kg body weight). Blood glucose was assessed every 15 minutes for 2 hours. One week later, glucose tolerance was assessed in a similar way (1.5u/kg lean mass). Insulin and glucose tolerance were then re-assessed after high fat diet feeding (~PND 140-160) (insulin dose 2.5u/kg lean mass, glucose dose 1.0u/kg lean mass)  
**Results**

**Discussion  
Conclusion**

References