**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). Measures of maternal health during pregnancy will be described separately. 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, offspring were weaned by sex and maternal feeding regimen and given normal chow diet (NCD) AL until 70 days of age.

*Maternal dietary treatment*

Dams were randomized to either early time-restricted feeding (eTRF) or *ad libitum* (AL) feeding during gestation (8= eTRF, 9=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 13-ZT 19) for the whole of pregnancy. Each day, all animals were transferred to a clean cage at ZT20, allowing for similar levels of handling of all experimental animals. After birth, all dams were switched to AL feeding of NCD and maintained on this diet until PND21.5. This meant that any phenotype in the offspring could be attributable to the gestational diet exclusively.

*Offspring growth and food intake monitoring*

Pups born to either eTRF or AL dams were weighed and counted within 24 hours of birth. Litters were reduced to 4 pups (2 male, 2 female when possible) at PND 3.5 to standardize milk supply among litters. Pups were weighed again at PND 7.5, 14.5, and 21.5. At PND 21.5, offspring were weighed, and body composition was assessed using EchoMRI before being weaned by sex and maternal feeding regimen and housed 4-5 per cage (eTRF males = 11, eTRF females = 19, AL males = 16, AL females =17). Offspring were given AL access to NCD until PND 70. Food intake and body composition were assessed weekly throughout the course of the experiment. Food intake is represented as a per animal per day average. After PND 70, all animals were switched to 45% High fat diet (research diets D12451, 4.73kcal/gram).

*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 was administered (Humulin, u-100; 0.75u/kg body weight). Blood glucose was assessed by glucometer every 15 minutes for 2 hours. One week later, glucose tolerance was assessed in a similar way (D-Glucose,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).

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### *Sacrifice and tissue collection:*

Offspring were sacrificed after the high fat diet glucose tolerance test (**Figure 1B**). Animals were fasted for 16 hours with *ad libitum* access to water. Animals were lightly anesthetized by isofluorane inhalation. Fasting blood glucose was determined by glucometer and a fasted blood sample was collected by retro-orbital bleed and immediately put on ice to clot. Once clot formed, whole blood was spun down in a cold centrifuge (4 degrees C) for 20 minutes at 5000 RCF. Serum was pipetted off and placed in the -80 degrees C freezer until analysis. After blood collection, animals were euthanized by isofluorane overdose and cervical dislocation. Animal body weight was measured immediately after euthanasia on an electronic scale to the nearest 0.1 gram. Liver, inguinal white adipose tissue (iWAT), gonadal white adipose tissue (gWAT), and quadriceps femoris muscle were dissected from the right side of each mouse and snap frozen in liquid nitrogen and stored at -80C until later use.

*Statistical analysis*

Repeated measures, such as body composition, weekly and cumulative food intake, and response to GTT or ITT were assessed via mixed linear effects modeling with random effects of mouse ID and dam and fixed effects of maternal dietary treatment, age, and sex. The models were separated by offspring diet (NCD or HFD) and analyses were sex-stratified to assess sexual dimorphism in phenotype. Static measures, such as fasting blood glucose, were assessed for linearity by Shapiro-wilk test and equivalence of variance by Levene’s test. Measures that were normal and of equal variance utilized student’s t-tests. Measures that were not normal used non-parametric Mann-Whitney tests. All measures whose p-values <0.05 were considered statistically significant.

**Results**

*Early Time Restricted Feeding (eTRF) has sex-specific effect in early life of offspring*

Body composition for offspring showed that eTRF males had similar body weights compared to age-matched AL counterparts (AL weight 0.14 grams less than eTRF males, p = 0.7, **Figure 2A**). Female AL offspring had higher body weights than age-matched eTRF females in the first 70 days of life (0.044 grams heavier, p = 0.004). A similar trend was observed for lean mass (**Figure 2C**), with females of eTRF dams 0.042 grams lighter than age-matched AL counterparts (p=0.0062), and males (0.012 grams heavier for eTRF vs AL, p=0.75). Although there were differences in body weight and lean mass observed in females, there were no differences in fat mass for either sex in the first 70 days of life (male AL 0.0069 grams lighter than eTRF males, p-value=0.30 ; female AL 0.0031 grams heavier than eTRF, p-value = 0.43, **Figure 2B**). Female offspring of AL dams consumed 0.529 kcals more per week than female eTRF offspring (p=0.049, **Figure 2D**). This difference in food intake was absent in male offspring (AL consumed 0.12 kcals less than eTRF, p=0.60). Cumulative effects of food eaten over the course of the first 70 days further demonstrated this sex-specific difference (**Figure 2E**). Male AL offspring consuming 0.64 fewer kcals per week than eTRF counterparts (p-0.34) and female AL offspring consuming 1.44 kcals fewer than eTRF counterparts (p=0.00022).

Insulin tolerance testing showed no interaction between time and maternal dietary treatment in males (**Figure 2F**). eTRF Males had blood glucose values 0.27 mg/dL lower than AL males at each timepoint(p=0.062). Female eTRF offspring also showed similar insulin tolerance compared to AL counterparts (0.27 mg/dL lower than AL females at each timepoint, p=0.88). Fasting blood glucose before administration of insulin also did not differ between males (AL = 197 vs eTRF = 215 mg/dL, p=0.20) or females (AL = 175 vs eTRF = 187 mg/dL, p=0.30). Glucose tolerance was similar in young adulthood between groups in both males and females (**Figure 2G**). There were also no statistically different responses to glucose tolerance testing (**Figure 2H**) in males (0.25mg/dL higher blood glucose per time point in AL vs eTRF, p=0.17) or females (0.27mg/dL higher blood glucose per time point in AL vs eTRF, p=0.79).

The effect sizes observed are modest and likely related to the large samples size. This led us to wonder how the offspring would behave given an overnutrition challenge; 45% of energy from fat. After 70 days, all animals were switched to AL access to 45% HFD. Weekly food intake and monitoring of body composition continued.

*Adult HFD Feeding generates glycemic deficits in a sex-specific manner*

After beginning high fat diet feeding, there were no distinct changes in body weights (**Figure 3A**). eTRF females had 0.013g lighter body weights than age-matched AL females (p=0.073). This was also similar to what was found in males, with no interaction of age and diet (eTRF males 0.014 grams heavier than AL males, p=0.70). There were also no effect on fat mass (**Figure 3B**) accretion in females(0.0078 grams lower fat mass vs AL, p=0.30), or males (0.013 grams lower fat mass vs AL, p=0.075); or lean mass (**Figure 3C**) in females(0.00012 grams higher fat mass vs AL, p=0.85), or males (0.0042 grams higher fat mass vs AL, p=0.28). Therefore, offspring of eTRF and AL made a similar transition to overnutrition challenge. Weekly food intake during HFD feeding were also similar among maternal feeding groups in males and females (**Figure 3D**)(females consuming 0.13 kcal per week less in AL vs eTRF group, p=0.76; males consuming 0.45 kcal per week more in AL vs eTRF group, p=0.57). Unlike the NCD period, females consumed similar cumulative kcals (**Figure 3E**) over the HFD period (AL had 1.21kcal per week greater intake vs eTRF, p=0.47). Males also showed no differences in cumulative food intake during HFD feeding (AL had 1.14 kcal per week greater intake vs eTRF, p=0.55).   
The first indications of differences in adult health outcomes between gestational eTRF and AL animals were in assessments of their glucose homeostasis. Insulin tolerance testing (**Figure 3F**) did not demonstrate further insulin sensitization (females 0.049 mg/dL higher glucose in AL vs eTRF, p=0.17; males 0.054mg/dL lower glucose in AL vs eTRF, p=0.85). There were also no difference in fasting blood glucose (**Figure 3G**) in males (260 AL vs 244 eTRF, p=0.40) or in females (AL 188 vs eTRF 203, p=0.20). During glucose tolerance testing (**Figure 3H**), males trended toward glucose intolerance (0.067 mg/dL higher glucose in eTRF compared to AL, p=0.14), which was absent in females (0.016 mg/dL higher glucose per time point in AL vs eTRF, p=0.61).

**Discussion**

To our knowledge, this is the first report off gestational time-restricted feeding effects on offspring in adulthood and after offspring high fat diet challenge. Although this effect fails to reach statistical significance, there is a trend toward having higher blood glucose in the initial response to glucose administration and lower blood glucose in the initial response to insulin administration.

**Conclusion**

References