# Abstract

# Introduction

* Maternal diet is important
* There is evidence that women are trying diets that are not vetted
* Review of maternal restriction and maternal eTRF
* Why it matters

# Methods

## Animal Husbandry

Age-matched (age in weeks) male and female C57Bl/6J mice were obtained from Jax. Animals were allowed to acclimatize to our facility for 1 week prior to beginning the experiment. Animals were maintained in a ventilated cages in a temperature and humidity-controlled room. In a 12:12 hour light dark cycle. 4 days before experimental treatment began, dams were single housed with extra enrichment. Every week, mice were weighed, and body composition was assessed using EchoMRI.

## Animal Dietary Treatment

Dams were randomized to either 24-hour access *ad libitum* (AL), or 6-hour early-time restricted feeding (eTRF) of standard laboratory chow (%P%F%C). the 6-hoour period mend that food was measured to the nearest 0.1 gram, then given in a hopper. We also measured the food in AL dam cages at ZT16. Animals were then allowed to eat freely for 6 hours. At ZT20, food was collected from the hopper and the bottom of the cage and measured again. Cages of all animals were changed at ZT20 to minimize food consumption of the bottom of the cage for eTRF dams and to have similar levels for handling stress in AL dams. Dams randomized to eTRF had empty hoppers placed in their new cages, and AL dams had their same hoppers replaced in their new cages. Food intake is presented in both 6-hour (ZT16-ZT20), and 24-hour intervals(ZT16-ZT16). Dams began dietary treatment

## Mating & Pups

After 6 days of diet, age and diet-matched males were introduced into female cages and were allowed to remain until copulatory plug was discovered (indicating pregnancy and gestational day E0.5). When pups were born, they were measured and counted within 24 hours, including those who were dead at birth. Pups were then left to nurse for 3 days. At postnatal day 3, litters were weighed then reduced to 4 pups to each dam (2 males, 2 females when possible) to standardize milk supply between litters. Pups were then reweighed on postnatal days 7, 14, and 21. At postnatal day 21dams and pups were sacrificed by Carbon Dioxide Inhalation and cervical dislocation.

## Intraperitoneal Insulin tolerance testing

Insulin tolerance was measured via an insulin tolerance test (ITT). On gestational day 16.5, dams were placed in a clean cage free of food with a water bottle at ZT20 (2AM). Dams were fasted for 6 hours. At ZT2, a fasted blood sample was collected via tail clip and handheld glucometer. After assessment of fasting blood glucose, an intraperitoneal injection of insulin (Humulin, 0.75mg/kg body weight) was given. Blood glucose following injection was taken every 15 minutes for 2 hours. Glucose area under the curve (AUC) was calculated by taking the sum of glucose values for each animal.

## Blood Collection and Hormonal Analysis

The day after the insulin tolerance testing, we collected blood samples from dams at ZT1 and ZT13. They were lightly anaesthetized via inhaled isoflurane then whole blood was collected via capillary tube and retroorbital bleed. Whole blood was left to clot on ice for 20 minutes, then was spun down in a cold centrifuge for 20 minutes at 2000G (Eppendorf, 4°C). Serum was pipetted off and stored at -80°C until later use. Insulin was assayed in serum using a commercially available ELISA kit (ALPCO, Cat number)

## Neonatal Life Outcomes

Gestational age was determined by the date of birth subtracted from date of copulatory plug. Litter size was represented as the number of pups (alive and dead) per dam, then averaged by feeding regimen. Percent survival was determined as the number of pups who were present at postnatal day 3 divided by the initial litter size times 100. Birth weight was calculated as the average of all living pups for each dam, then further averaged by feeding regimen.

## Statistical Analyses

Values are represented as mean ± standard error whenever possible. Pairwise values are evaluated by Shapiro test for normality and Levene’s Test for equivalence of variance. When values were normal and of equivalent variance, Student’s T Test was used, if they were not normal, then we used the appropriate non-parametric test. For repeated measures, such as food intake, and body composition, linear mixed effect modeling was completed using LME4 (1). We used random effect of maternal ID and dam ID and fixed effects of feeding regimen, day of gestation, and sex (for pup analyses).

# Results

# Discussion

# References

1. **Bates D**, **Mächler M**, **Bolker B**, **Walker S**. Fitting Linear Mixed-Effects Models Using lme4. *J Stat Softw* 67: 1–48, 2015. doi: 10.18637/jss.v067.i01.