# Outline

Early Time Restricted Feeding Does not Alter Food intake or Gestational Weight Gain

* There is a ~7-day adaptation period to eTRF
* Food intake is greater for eTRF dams at 6 hours, but not 24 hours
* eTRF does not cause weight loss during pregnancy

Insulin Responsiveness is Similar in eTRF Dams, but There is a More Robust Rebound from Hypoglycemia

* Insulin tolerance is similar but there is a more severe rebound of glucose after drop in eTRF dams
* Will do insulin elisa \*insulin levels are XXX in eTRF

Fecundity, birthweights and growth are similar between control and eTRF pregnancies

* similar latency/fertility
* No differences in GA or birthweight
* Significant reductions in litter size and 3-day survival
* Growth to PND 21

# Abstract

# Introduction

Dietary health during pregnancy has long been a topic of intense research interest. Since the early days of the developmental origins of health and disease (DOHaD) hypothesis when Dr. David Barker proposed that *in utero* conditions could program the resultant child for health or disease, based on the mismatch they would face once born (1). The most prominent of these studies children who were *in utero* during extreme famine during the “Dutch Hunger Winter” during the second world war. Finding that times of dramatically reduced food intake during pregnancy could impart higher risk for cardiometabolic risk in adulthood, even if risk ratios were adjusted for infant birthweights (2, 3). Since that time, studies seek to understand the role of adverse nutrition in the womb and its impacts on children once they are born and even well after having reached adulthood.

For obvious ethical reasons, much work in DOHaD has been adapted to preclinical models of pregnancy. Poor nutrition in pregnancy is often accomplished in animal model through means of calorie restriction, protein restriction, or uterine artery ligation. MORE HERE Such studies often, but not always, find that pups born to dams who experienced restriction of some sort during pregnancy are smaller. When pups are followed to later stages of life, like adolescence and adulthood, there can be metabolic and body composition alterations, such as increased adipose tissue. glucose intolerance, or insulin resistance.

There is evidence to suggest that timing of food intake is an important, yet critically understudied aspect of nutrition during pregnancy. There are few models of time-restricted feeding in pregnant rodents in the scientific literature. These projects find that time-restricted feeding of high fat, high sucrose diets in rodents can reduce oxidative stress in placental tissues that results from overnutrition(4), and improve fetal lung development compared to *ad libitum* fed high fat, high sucrose dams (5). There is also evidence that estrus cyclicity and follicle development that can occur with poor nutrition are rescued with TRF of HFHS feeding compared to *ad libitum* HFHS(6). Two studies have found that TRF during pregnancy has impact for insulin homeostasis in adulthood. One finding that glucose intolerance on chow in adult offspring from eTRF dams (7), and another from our group finding that glucose intolerance only occurs in male offspring after long term high fat, high sucrose feeding (11). As the majority of the attention that has been paid to this dietary manipulation focuses on resultant offspring, scientist have failed to characterize the effects of TRF during the course of the pregnancy in the dam.

Although preclinical work is limited, there is evidence that those who are currently pregnant or considering pregnancy would consider manipulation of the timing of food intake as a modality to improve health. Flanagan and colleagues asked about attitudes of trying time-restricted eating during the course of pregnancy, 24.7% of those polled said they would be open to trying a time-restricted regimen during the course of pregnancy to improve their health (9). There was also a qualitative response from one participant who stated they had practiced intermittent fasting during their pregnancy, after finding out they were 9-weeks pregnant while already following this diet. Recently, a case study of manipulation of the feeding window and reducing meal numbers to manage gestational diabetes reduced postprandial blood glucose when dietary quality manipulation and exercise was insufficient in gaining control of GDM (10). Although epidemiological work on the timing of eating is still limited in pregnant populations, an association between prolonged overnight fasting and fewer meals during the day has been found with a more favorable maternal glycemic response in the second trimester of pregnancy (11). There have also been studies that suggest that eating overnight, although somewhat common, can be associated with poorer pregnancy birth outcomes (12).This suggests that there is evidence that human pregnant populations either practice or consider practicing this diet and that we have limited understanding of its implications for safety or efficacy in improving perinatal health.

In light of the potential use of this diet to improve health during pregnancy and limited characterization of the practice in pregnant populations on the parent, we sought to identify the effect of early time-restricted feeding (eTRF) on maternal insulin sensitivity and early postnatal health in resultant offspring using a mouse model. We hypothesized that maternal glycemic health would be improved through eTRF of normal chow and that resulting offspring would not be adversely affected.

# 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 (24% Protein, 5% Fat, 35.7%Carbohydrate). 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 determined 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. Rates of initial reduction in blood glucose was calculated by limiting the data to 45 minutes after injection. We then modeled the exponential rate of decay in blood glucose for each dam as a slope and took the average by feeding group. We also calculate the rate of rebound after hypoglycemia by limiting the data to data collected 75-120 minutes after injection, then modeling the linear rise in glucose as a rate of time\* group, then averaging by feeding group.

## 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 delivered 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. 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. 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 (13). We used random effect of maternal ID and dam ID and fixed effects for feeding regimen, day of gestation or postnatal age, and sex (for pup analyses).

# Results

## Early Time Restricted Feeding Does not Alter Food intake or Gestational Weight Gain\

In order to characterize the effects of early time-restricted feeding (eTRF) during pregnancy, we used a mouse model. Dams were randomized to eTRF between ZT16-ZT20 or *ad libitum* (AL) feeding of laboratory chow (**Figure 1A**)(8). After one week acclimating to the diet (RESULT ABOUT ADAPTATION TO DIET), males were added to the cage and checked daily until a copulatory plug was discovered. Dams were kept on respective diets until they gave birth, at which point they were all switched to AL access to chow (**Figure 1B**).

## Insulin Responsiveness is Similar in eTRF Dams, but There is a More Robust Rebound from Hypoglycemia

To test our hypothesis of improved glycemic health during pregnancy for dams fed eTRF, we conducted and intraperitoneal insulin tolerance test (ITT) on gestational day 16 (**Figure 3A**). We found that fasting blood glucose was similar between eTRF and AL dams at the beginning of the ITT, (**Figure 3B**, p=0.27). Using linear mixed effect models with a random effect for dam ID and fixed effects of time and maternal dietary regimen, we found there was a significant interaction between time and maternal diet (**Figure 3A**). eTRF dams tended to have 0.35 ±0.07 mg/dL greater glucose at each time point than AL dams (pdiet\*time <0.001). There was a 19.8% greater area under the curve in eTRF dams (**Figure 3C**, p=0.03). To assess the initial response to insulin administration, we evaluated the rate of glucose drop in the first 30 minutes after the insulin injection. We found eTRF dams and AL dams to be similarly responsive, with comparable rates of glucose drop (**Figure 3D**, p=0.75). We noticed that eTRF dams seemed to have a greater rate of glucose recovery after reaching their lowest glucose value, so we evaluated the difference in the rates of glucose recovery after hypoglycemia by using a linear model for each group in the last 60 minutes of the experiment. We found that eTRF dams recovered glucose at a rate 2.4% faster than AL dams, but this did not reach statistical significance (**Figure 3E**, p=0.084). INSERT INSULIN ELISA HERE. These data suggest that insulin sensitivity is similar to normal pregnancies in AL fed dams, but that there is a more robust response to hypoglycemia in dams who undergo chronic, prolonged overnight fasts during the perinatal period. This results in a glucose values that surpass their baseline glucose values.

## Fecundity, birthweights and growth are similar between control and eTRF pregnancies

To evaluate the effect of gestational eTRF on reproductive outcomes that are similarly observed and often impacted by gestational food restriction, we observed measures for litter size, average rates of survival during postnatal days, and weights of pups in the first 24 hours of life. We calculated gestational age for each dam as the average number of days between copulatory plug discovery and parturition. We found that eTRF and AL dams had similar gestational ages within anticipated normal range for mouse pregnancy (**Figure4A**, p=0.201). There was a 28.6 percent reduction in the number of pups surviving to PND3 in eTRF litters (**Figure 4B**, p=0.039). Litter sizes were 15.3 percent smaller in eTRF dams: though this did not reach statistical significance (**Figure 4C**, p=0.072). Despite smaller litter sizes in eTRF dams, the average weight of each pup was similar between maternal dietary treatments (**Figure 4D**, p=0.13). This suggests that there may be adverse effects for dams fed eTRF, who may cannibalize their pups at greater rates, resulting in worse survival. However, there is no evidence of overt restriction when we look at birth weights.

we assess the latency to copulatory plug appearance after pairing. We found that.

# Discussion

Hua and colleagues found increase pups per litter in both HFHS and CD

* We didn’t look at latency in all 3 cohorts – could talk about % of failed mating though, if appropriate.

Reduced litter size and reduced survival – could be secondary to pups being cannibalized before we count them?

# References

1. **Barker DJ**, **Osmond C**. Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. *Lancet Lond Engl* 1: 1077–1081, 1986. doi: 10.1016/s0140-6736(86)91340-1.

2. **Roseboom TJ**, **Meulen JHP van der**, **Osmond C**, **Barker DJP**, **Ravelli ACJ**, **Schroeder-Tanka JM**, **Montfrans GA van**, **Michels RPJ**, **Bleker OP**. Coronary heart disease after prenatal exposure to the Dutch famine, 1944–45. *Heart* 84: 595–598, 2000. doi: 10.1136/heart.84.6.595.

3. **Rooij SR de**, **Painter RC**, **Phillips DIW**, **Osmond C**, **Michels RPJ**, **Godsland IF**, **Bossuyt PMM**, **Bleker OP**, **Roseboom TJ**. Impaired Insulin Secretion After Prenatal Exposure to the Dutch Famine. *Diabetes Care* 29: 1897–1901, 2006. doi: 10.2337/dc06-0460.

4. **Upadhyay A**, **Anjum B**, **Godbole NM**, **Rajak S**, **Shukla P**, **Tiwari S**, **Sinha RA**, **Godbole MM**. Time-restricted feeding reduces high-fat diet associated placental inflammation and limits adverse effects on fetal organ development. *Biochem Biophys Res Commun* 514: 415–421, 2019. doi: 10.1016/j.bbrc.2019.04.154.

5. **Upadhyay A**, **Sinha RA**, **Kumar A**, **Godbole MM**. Time-restricted feeding ameliorates maternal high-fat diet-induced fetal lung injury. *Exp Mol Pathol* 114: 104413, 2020. doi: 10.1016/j.yexmp.2020.104413.

6. **Hua L**, **Feng B**, **Huang L**, **Li J**, **Luo T**, **Jiang X**, **Han X**, **Che L**, **Xu S**, **Lin Y**, **Fang Z**, **Wu D**, **Zhuo Y**. Time-restricted feeding improves the reproductive function of female mice via liver fibroblast growth factor 21. *Clin Transl Med* 10: e195, 2020. doi: 10.1002/ctm2.195.

7. **Prates KV**, **Pavanello A**, **Gongora AB**, **Moreira VM**, **de Moraes AMP**, **Rigo KP**, **Vieira E**, **Mathias PC de F**. Time-restricted feeding during embryonic development leads to metabolic dysfunction in adult rat offspring. .

8. **Mulcahy MC**, **Habbal NE**, **Snyder D**, **Redd JR**, **Sun H**, **Gregg BE**, **Bridges D**. Gestational Early-Time Restricted Feeding Results in Sex-Specific Glucose Intolerance in Adult Male Mice. bioRxiv: 2022.04.27.489576, 2022.

9. **Flanagan EW**, **Kebbe M**, **Sparks JR**, **Redman LM**. Assessment of Eating Behaviors and Perceptions of Time-Restricted Eating During Pregnancy. *J Nutr* 152: 475–483, 2022. doi: 10.1093/jn/nxab397.

10. **Ali AM**, **Kunugi H**. Intermittent Fasting, Dietary Modifications, and Exercise for the Control of Gestational Diabetes and Maternal Mood Dysregulation: A Review and a Case Report. *Int J Environ Res Public Health* 17: 9379, 2020. doi: 10.3390/ijerph17249379.

11. **Loy SL**, **Chan JKY**, **Wee PH**, **Colega MT**, **Cheung YB**, **Godfrey KM**, **Kwek K**, **Saw SM**, **Chong Y-S**, **Natarajan P**, **Müller-Riemenschneider F**, **Lek N**, **Chong MF-F**, **Yap F**. Maternal Circadian Eating Time and Frequency Are Associated with Blood Glucose Concentrations during Pregnancy. *J Nutr* 147: 70–77, 2017. doi: 10.3945/jn.116.239392.

12. **Loy SL**, **Loo RSX**, **Godfrey KM**, **Chong Y-S**, **Shek LP-C**, **Tan KH**, **Chong MF-F**, **Chan JKY**, **Yap F**. Chrononutrition during Pregnancy: A Review on Maternal Night-Time Eating. *Nutrients* 12: 2783, 2020. doi: 10.3390/nu12092783.

13. **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.