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# **Aim 3: Determine the effect of** **early time-restricted feeding in the perinatal period on offspring survival, growth, and metabolic health.**

## Background:

### Childhood origins of metabolic disease

* Introduction of DOHaD
  + Dutch hunger winter
  + Studies of nutrient restriction in gestation in animal models have demonstrated that caloric restriction increases incidence of low birthweight, and may initiate unhealthful catch up growth upon weaning, resulting in excess body weight, body fat, and leptin resistance upon reaching adulthood(Ross & Desai, 2005).
  + Organogenesis/2-hit hypothesis

### Early life exposure to time restricted feeding

Early life is characterized by rapid rates of growth and differentiation and furthermore is a critical period for programming propensity for dysmetabolism. There is substantial evidence that gestation is a critical time for future offspring health. Studies in humans have demonstrated that exposure to food shortage/famine can Further evidence has demonstrated that the immediate post-natal life and time preceding adulthood are also crucial in determining risk of ill health in adult life. The largest literature of maternal time-restricted feeding in pregnancy exists in women fasting in observance of Ramadan during their pregnancies. These studies show that gestational age is often similar between those who fasted and those who did not fast during pregnancy. Furthermore, there may be a greater incidence in low birth weight babies, especially if the Ramadan fasting took place in the first trimester of pregnancy. However, it is my belief that Ramadan fasting is not a good proxy for TRF during gestation, as it may better model of food entrained chronodisruption during gestation, which has been shown by Salazar and colleagues to be detrimental to disrupt glucocorticoid stress signaling in rat fetuses, thereby altering their propensity to develop metabolic disease(Salazar et al., 2018).

#### Early Post-natal Time-Restricted Feeding

In the field of DOHad, the early parts of life extend beyond the gestation period and extend into the early post-natal life. Time-restricted feeding has been evaluated in the early postnatal period in one study, in hope it would mitigate the development of obesity later in life. This study began 8-hour, dark cycle TRF immediately after weaning and kept pups on this schedule for 4 weeks. After 4 weeks, they were switched to AL feeding. Instead of the typical protective effects often seen in TRF in adult populations, harmful metabolic effects were noted. Among them are hyperglycemia, reduced size and area of pancreatic islets, reduced insulin production, increased fatty liver, reduced immune competency, and delayed pubertal maturation(Hu et al., 2019). This suggests that there are effects of TRF in the development period. However, the early post-natal life is distinct from the gestational period; as it is the time for behavior, brain, and development, as opposed to the main time of tissue accretion, organogenesis and \_\_\_ that gestation is(CITE). Therefore, post-natal TRF effects are unlikely to be the same as those during gestation.

#### Gestational Time Restricted Feeding

One work has been completed in gestational eTRF. This focused on HFD-TRF feeding in comparison to HFD-AL feeding. This paper focused on in utero and maternal general habitus, and failed to

Upadhyay and colleagues demonstrated the TRF of HFD could be protective compared to AL HFD feeding on fetal development, with a normalization of placetal:fetal ratio, lower liver TG, and improved lung maturity in TRF fed fetuses at E18.5. This suggests that TRF is able to abrogate the effects of high fat diet feeding in utero. It would be worthwhile to see the effects of TRF-NCD. However, the post-natal period, including birth indices, survival, growth, and metabolic health were not evaluated in this study, therefore eTRF effects on the offspring have yet to be characterized in the literature.

## Specific aim 3.1 Will dam eTRF during gestation reduce pup survival?

* Canonical studies of food restriction report that with caloric restriction during gestation, there is \_\_\_\_ effect on survival of the pups(CITE).
* Overall… maternal killing

Some studies use food deprivation as a means to induce stress in maternal animals. This may be enough to impact survival of the pups.

* Sex differences may exist in the survivorship of offspring. Work done in dairy cows has demonstrated that restrictive feeding practices initiated before mating resulted in smaller calves, and fewer female calves surviving compared to AL fed controls(Vinsky, Novak, Dixon, Dyck, & Foxcroft, 2006). However, the majority of animal models find that TRF rarely induces caloric deficit when compared to AL fed controls (CITE ALL THESE). In order to assess survival of the pups, animals will be counted on PND 0.5 and sexed as soon as possible. This was not something we evaluated, but we will in the future be sexing and recording weights of all fetuses before culling to even litters. This will better enable us to detect any sex-specific differences in early post-natal survival or in birth numbers.
* In utero – cannot find that paper about GC and selective survival.

## Specific aim 3.2 Will gestational exposure to eTRF reduce duration of gestation or birthweight?

Birthweight is an important indicator of offspring and placental health (CITE). It is often seen that birthweights in nutritionally restricted pregnancies are more likely to be lower than normally fed pregnancies. It is also seen that timing of restriction may play a particularly prominent role in determining risk of low birth weight. Fetuses exposed to the Dutch hunger winter early during gestation had lower birth weights, but those who were exposed during late gestation have normal birthweights(Schulz, 2010). This has been reinforced by some studies that evaluate Ramadan fasting during gestation, with only early gestation fetuses exposed to fasting having greater propensity for reduced birthweight(Ziaee et al., 2010). Although total nutrient restriction and daytime fasting are not good models of TRF, as they either reduce total number of calories or introduce a disruption to the natural circadian cycle for eating. The one study of gestational TRF by Upadhyay and colleagues demonstrated that HFD-TRF feeding during pregnancy not only failed to impart differences in birthweight, but was actually able to correct increase compared to AL-HFD feeding. (Upadhyay et al., 2019). Therefore, it is my hypothesis, that because of the similar food intake present in eTRF dams, compared to AL dams, that birthweight will be similar between maternal feeding groups. To determine this, weights will be taken of each pup on day PND 0.5.

The preliminary cohort data demonstrates that average birthweight per pup does not differ between maternal feeding groups. One possibility that we had not considered during the first cohort, is that eTRF may alter birthweight in a sex-specific manner. Previously, we d

* BDNF crosses the placenta
* Sex diff
* We did not look into sex specific reductions in birthweight, but found that in general, birthweight was equal between eTRF and AL litters. Therefore, it is unlikely that large differences exist.

## Specific aim 3.3 Will gestational exposure to eTRF alter food intake and growth trajectory?

Less significant induction of the integrated stress response suggests that he in utero environment is not one that is inhospitable to fetuses, but may be one that is slightly stressed, as the ISR was moderately upregulated compared to NCD-AL feeding(Upadhyay et al., 2019).

* BC, weight, food intake
* Limiting litters

One existing study to evaluate TRF in developing animals discovered that TRF in the post weaning life, even when followed by 4 weeks of AL feeding, elicited severe metabolic dysfunction in mice(Hu et al., 2019). This was not improved by continued TRF feeding and manifested with a chow diet. The TRF animals demonstrated hyperglycemia, insulinemia, and impaired liver function, reduced immune response, altered gut microbiome, and delayed sexual maturation(Hu et al., 2019).

Growth encompasses many factors including the trajectory of body composition, the propensity for food intake and energy expenditure, and of maturation.

## Specific aim 3.4 Will gestational exposure to eTRF confer metabolic benefit when challenged with a high fat diet?

* BC
* If D then why
* I hypothesize that eTRF animals will have resistance to DIO because \_\_\_\_. To test this, at adulthood (70 days of age) all offspring will be switched to a 45% HFD. This diet treatment will remain for 10-12 weeks. Weekly measurements of body weight, fat mass, lean mass, and food intake will be assessed.
* The hypothesis is that in response to a HFD, TRF exposed mice will be more resistant to diet induced obesity, manifesting as a lower body fat percentage. It is also entirely possible that HFD will be poorly received as it does not match the utero environment, making TRF exposed mice more likely to be hyperglycemic or fat.
* WHY WOULD THEY BE RESISTANT TO A HFD?
  + It could be that because they had no exposure to this type of feeding/nutrient levels in the womb, that they are particularly susceptible to HFD-induced metabolic disease.

## Specific aim 3.5 Will gestational exposure to eTRF improve insulin sensitivity and glycemia of offspring?

The many studies in humans and in animals of TRF demonstrate a consistent propensity for improvement in insulin and glucose homeostasis. For this reason, I expect that offspring born to dams fed eTRF will develop more sensitive to insulin and glucose. However, reduced insulin production, as is often seen in TRF papers, concomitant with the process of organogenesis of the metabolically active organs (pancreas, liver).

* Think about hormones/signals that are increased by fasting and look into the periods of organogenesis---this has to have been done before.
* ITT at NCD/HFD why not GTT
* Why, cond/ obesity
* Taking blood and tissues
  + Fed and fasted blood – insulin/glucose/gut signaling peptides
  + Liver
  + Pancreas
  + Iwat
  + Gwat
  + Muscle (one glycolytic, one oxidative)
  + NTS/AP – for GDF15 signaling

## Methods:

### Animal care and use:

Upon birth, litters were counted and individual pups weighed within 24 hours. At postnatal day 3, litters were reduced to four (two males and two females, when feasible) to standardize milk supply. At 21 days, pups were weaned by sex and maternal treatment group. Upon weaning, animals are allowed 24-hour access to chow (5% fat, 24% protein, 3.7% sucrose, 32% starch, 2.91 kcal per gram) and water.

### Body composition:

Body weight was assessed using a scale (). This was assessed at birth, 7, 14, and 21 days of life. At 21 days of life, weekly indirect body composition assessment using EchoMRI; fat mass, lean mass, and free water were determined in addition to body weight.

### Survival:

Survival of pups will be assessed by counting the number of pups in each litter each day until PND 3.

### Determination of sex:

In order to determine sex, at PND3, anogenital distance of each pup will be evaluated. Those pups with greater anogenital distances will be designated male, and those with lesser distances, female. This will be confirmed by genotyping the fetal tissue for expression of SRY, which is carried on the Y chromosome and is causal in phenotypic sexual determination (Larney, Bailey, & Koopman, 2014).

### Reduction of litters:

Because maternal milk supply may differ based on number of pups, milk supply will be standardized after the initiation of the lactational period. At PND 3, litters will be reduced to 4 when possible (2 male, 2 female). This will help to ensure each dam can supply sufficient and equal amounts of milk to each pup.

### Food intake:

Food intake monitoring began at weaning. Weekly food intake was measured in grams for each cage, and food intake in calories was computed by taking the total food intake per week and dividing by number of animals in each cage. At 65 days of age, animals were switched to *ad libitum* feeding with high fat diet (HFD) (45% fat, 20% protein, 17% sucrose, and 7% starch, 4.73 kcal per gram). Animals will remain on HFD for 10 weeks.

### Insulin Sensitivity:

*Insulin tolerance test:*

After 6-hour fast, blood glucose was taken using a glucometer and tail clip. Animals were given intraperitoneal 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 PBS was administered, the animal was then removed from the experiment, and subsequent blood glucose measurements were omitted from data analysis.

### Statistical Analyses:

All statistical analyses were completed in R (version \_\_\_\_\_). Repeated measures, such as body weight, body composition, food intake, and insulin tolerance testing utilized mixed linear modeling (LME4 package) with each animal assessed as a random effect. All models were tested for sex-interaction. Models were built bottom up and were tested in pairs using ANOVA. Models where ANOVA p value was <0.05 were considered statistically significant.

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**Determine the effect of** **early time-restricted feeding in the perinatal period on offspring health.**