# Gestational early time-restricted feeding results in mild maternal glycemic differences, reduced litter sizes, and pup survival

# Abstract

Time-restricted Feeding is an increasingly common diet that may modulate metabolic health. As recent evidence suggests pregnant people have considered or used the diet and pregnancy is a critical period of development for both parent and child, investigation of the effects of TRF during pregnancy in preclinical models is warranted. We employed a chow fed daily early time-restricted feeding (eTRF) regimen during the dark during the course of mouse pregnancy and evaluated its effect on maternal body weight and food intake, maternal insulin sensitivity, and offspring early postnatal health. We found that dams who were fed eTRF consumed similar kilocalories during the course of their pregnancies and gained comparable weight to Ad Libitum dams. Dams who were exposed to eTRF had similar insulin sensitivity but increased rebound from hypoglycemia during late gestation. Fertility, and pup birth weight were comparable between diets, but there was a significant reduction in litter sizes and rates of survival to postnatal day 3 in eTRF dams. Pups born to eTRF dams had similar growth to postnatal day 21. These data suggest that eTRF during gestation has mild effects in pregnant populations but may impact offspring health. More evaluation of this phenotype is warranted in order to translate to pregnant human populations.

# Introduction

The timing of eating with respect to one’s circadian rhythm has become an area of interest as a modifiable component of the diet to alter for health reasons. There are many forms of eating that attempt to manipulate the timing of food, among them is time-restricted eating or feeding (TRF/TRE). With this modality, one confines caloric intake to a predictable and condensed period of time each day, in line with the circadian day, ultimately increasing the number of hours spent fasting.

Preclinical models have thoroughly detailed this dietary manipulation. Often, when TRF is employed in rodents provided with a high-fat, high-sucrose diet, that weight gain is reduced compared to ad libitum fed controls (Boucsein et al., 2019; Chaix et al., 2019; Sherman et al., 2012). More importantly, TRF in rodent models on has been shown to positively impact glucose homeostasis in rodent models (Chaix et al., 2019; Chung et al., 2016; Hatori et al., 2012; She et al., 2021; W. Wang et al., 2021; X.-P. Wang et al., 2020; Wilson et al., 2020; Woodie et al., 2018), although this is not present in all studies (Das et al., 2021; García-Gaytán et al., 2020; Sherman et al., 2012). The focus of this body of literature is the ability of TRF to protect from high-fat, high-sucrose diets in adult animals, with few groups working on younger populations or focusing on the effects during critical periods of development.

Human models have evaluated this as a method to treat or prevent accumulation of deleterious amounts of adipose tissue which may result in metabolic illness. Although weight loss is often modest (Gabel et al., 2018; Hutchison et al., 2019; Lowe et al., 2020; Moro et al., 2016; Stote et al., 2007), there have been comparable health improvements in those with controlled periods of time-restricted eating; such as reductions in blood pressure (Gabel et al., 2018; Jamshed et al., 2019; Sutton et al., 2018), improved measures of oxidative stress (Moro et al., 2016; Sutton et al., 2018), reductions in glycemic excursions (Jamshed et al., 2019), or alterations in insulin indices (Hutchison et al., 2019; Jamshed et al., 2019; Moro et al., 2016; Sutton et al., 2018). Some have even found improvement without weight loss (Sutton et al., 2018). Currently, the focus of the majority of TRF/TRE studies have been in preventing or lessening metabolic effects from over-feeding in adults, leaving critical periods of development and lower-calorie diets without evidence. Furthermore, as the popularity of this diet increases, there are critically important populations that develop lasting effects from attempting this diet before its effects are fully characterized; o such population is those who are attempting to become or are pregnant.

Dietary health during pregnancy has long been a topic of intense research interest. This research intensified when 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 (Barker & Osmond, 1986). Ultimately, these studies were the first of the developmental origins of health and disease (DOHaD) field. The most prominent DOHaD study examined children who were *in utero* during extreme famine during the “Dutch Hunger Winter” during the second world war. Finding that times of food restriction during pregnancy could impart higher risk for cardiometabolic risk in adulthood, even after risk ratios were adjusted for infant birthweights (Heijmans et al., 2008; Rooij et al., 2006; Roseboom et al., 2000). Since that time, many projects seek to understand the role of adverse nutrition in the womb and its impacts on children, even well after having reached adulthood.

There is evidence to suggest that timing of food intake is an important, yet critically understudied aspect of nutrition during pregnancy. Some of this evidence comes from models of time-restricted feeding in pregnant or reproductively active rodents. These studies find that time-restricted feeding of high-fat, high-sucrose diets in rodents can reduce oxidative stress in placental tissues that results from overnutrition during pregnancy (Upadhyay et al., 2019), and improve fetal lung development compared to *ad libitum* fed high-fat, high-sucrose dams (Upadhyay et al., 2020). There is also evidence that estrus cyclicity and ovarian follicle development that can occur with overnutrition are rescued with TRF of HFHS feeding compared to *ad libitum* HFHS (Hua et al., 2020). Existing studies in rats have found that TRF during pregnancy has impact for insulin homeostasis in adulthood. In adult offspring of eTRF dams, glucose intolerance developed on a chow diet (Prates et al., 2022), and another from our group finds that glucose intolerance only occurs in male offspring after long term high fat, high sucrose feeding (Mulcahy et al., 2022). Still others have sought to replicate TRF with chronodisruption (as a proxy for Ramadan fasting) and growth restriction was present on a chow diet, where dams ate fewer calories, gained less weight, and pups were smaller in litters randomized to TRF during the light cycle (Alkhalefah et al., 2021a). As the majority of the attention that has been paid to this dietary manipulation focuses on resultant offspring either as adults or in the fetal stage, scientists have failed to comprehensively characterize the effects of TRF during the course of the pregnancy in the dam without chronodisruption as part of the model.

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 participants about their attitudes toward trying time-restricted eating during the course of pregnancy. Of those polled, 24.7% said they would be open to trying a time-restricted regimen during the course of pregnancy to improve their health (Flanagan et al., 2022). There was also a qualitative response from one participant who stated they had practiced intermittent fasting during their pregnancy. Recently, a case study also identified manipulation of the feeding window and reducing meal numbers to manage gestational diabetes (Ali & Kunugi, 2020). 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 (Loy et al., 2017). Eating overnight, although somewhat common during pregnancy can also be associated with poorer birth outcomes (Loy et al., 2020).

The most robust literature in humans that explores maternal dietary restriction during gestation are studies that evaluate pregnancy outcomes after religious observance of Ramadan in Muslim pregnant populations. Such studies have found that observing Ramadan fasting during pregnancy does not result in reduced gestational age at delivery (Awwad et al., 2012; Safari et al., 2019), does not impact birth weight (Glazier et al., 2018; Safari et al., 2019), and in some studies reduced odds of developing gestational diabetes (Awwad et al., 2012; Daley et al., 2017; Safari et al., 2019). However, Ramadan is an imperfect proxy for TRF, as altered timing of eating is concomitant with sleep disruption and dietary quality changes. Therefore, more direct analyses of altered timing of eating are warranted. 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 impacting 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 (17±0.072 weeks) male and female C57BL/6J mice were obtained from The Jackson Laboratories (RRID:IMSR\_JAX:000664). 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 Intervention

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 ZT14. 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 (ZT14-ZT20), and 24-hour intervals (ZT14-ZT14). Dams began dietary treatment

## Estrus Testing

To understand how eTRF affects estrus cycle health, we monitored the estrus stage of females after randomization to dietary treatment each day until copulatory plug appeared in cohort 2. One hour before food was given (ZT13), a vaginal canal smear was collected for each dam. Using a p20 pipette, 15uL of sterile PBS was lavaged into the vaginal canal and mixed by plunging up and down briefly. Then the same pipette was used to recollect as much of the 15 uL volume as possible which was immediately transferred to a microscope slide. While still wet, slides were visualized at 10X magnification and images were captured. If the sample was dense, dry, or had crystals, more PBS was added and mixed gently with a clean pipette tip. Cell type and proportions were examined and stages were assigned based on methods described in (Caligioni, 2009; McLean et al., 2012). We calculated the total number of days in each stage for each dam, then averages were taken for each maternal dietary regimen.

## Matin, Fertility & 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). To assess fertility, latency from mating to plug and rates of successful mating events were calculated. 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 determined 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 time:treatment interaction.

## 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 , ultra-sensitive mouse ELISA kit (Crystal Chem, catalog #90080).

## 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 estimated as 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 fertility measures (estrus staging and success of mating events), chi-square analyses were completed, comparing the proportion of days distributed among estrus stage by maternal dietary treatment, assuming an equal distribution as between stages. For repeated measures, such as food intake, and body composition, linear mixed effect modeling was completed using lme4 (Bates et al., 2015, p. 4). 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 nor Gestational Weight Gain

In order to characterize the effects of early time-restricted feeding (eTRF) during pregnancy, we randomized dams to eTRF between ZT14-ZT20 or *ad libitum* (AL) feeding of laboratory chow (**Figure 1A**)(Mulcahy et al., 2022). After one week acclimating to the diet, males were added to the cage and examined daily until a copulatory plug was identified. Dams were kept on respective timed diets until they gave birth, at which point they were all switched to AL access to chow (**Figure 1B**). During the first week following randomization, there was an evident period of adaptation, where eTRF dams slowly increased their 6-hour food intake by 1.15±0.32 kcals per day as they habituated to reduced food access time. This resulted in a significant interaction between day of exposure and maternal dietary regimen (**Figure 2A**, pday:diet=0.00033). Using linear mixed effect models, we found that in the pre-pregnancy period, eTRF dams consumed 6.63 ± 1.59 more kilocalories during their 6-hour feeding period than AL dams did (**Figure 2B,** p<0.001). There was a significant interaction between gestational age and maternal dietary regimen during pregnancy, where eTRF dams consumed significantly more food at 6 hours during pregnancy, but this difference increased as gestational age advanced (**Figure 2C**, pdiet:gest.age=0.001). However, when we examined 24-hour intake, we found that during both the pre-pregnancy and pregnancy periods, eTRF dams consumed similar kcals compared to AL dams (**Figure 2D**, pdiet = 0.66 and **Figure 2E**, pdiet = 0.72). Consistent with their matched food intake, dam body weights remained comparable during pre-pregnancy (**Figure 2F**, p=0.68) and pregnancy (**Figure 2G**, p=0.34). These data suggests that after an adaptation period, dams randomized to eTRF during the perinatal period are able to maintain normal caloric intake and maintain appropriate body weights for pregnancy.

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

To test whether dams fed eTRF had improved insulin responsiveness, we conducted intraperitoneal insulin tolerance tests (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 that eTRF dams averaged 17.6±12.6 mg/dL greater glucose at each time point than AL dams during the course of the full 120 minutes (pdiet\*time <0.001; **Figure 3A**). As such there was a 19.8% greater area under the curve in eTRF dams (**Figure 3C**, p=0.03) indicating insulin insensitivity. To probe this further, we assessed the initial response to insulin administration. We found eTRF dams and AL dams to be similarly responsive in the initial stages, with comparable rates of glucose drop (**Figure 3D**, p=0.75). eTRF dams seemed to have a more rapid glucose recovery after reaching their lowest glucose value. We evaluated the difference in the rates of glucose recovery after hypoglycemia by constructing linear models for each group in just the last 60 minutes of the experiment. We found that eTRF dams recovered glucose at a rate 2.4-fold faster than AL dams, but this did not reach statistical significance (**Figure 3E**, p=0.084). Despite the significant difference in response to ITT, there were no significant differences in serum insulin concentration between maternal feeding regimens at ZT1 or at ZT13 (**Figure 3F**, p=0.38). 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 change is unlikely to be driven by baseline differences in insulin concentration.

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

We assessed fertility by evaluating the time spent in each stage of the estrus cycle, the latency to copulatory plug appearance after pairing, and rate of successful pairings. We found that the average number of days spent in each estrus stage was similar despite the dam undergoing eTRF (**Figure 4A**, p=0.70). The latency to copulatory plug was less than one day longer (2.29 vs 2.94, AL vs eTRF respectively) in eTRF dams (**Figure 4B**, p=0.39). When comparing mating pairs who were successful and had litters to those that did not, there was no difference in the rates of pregnancy between feeding regimens (not pictured, p=0.99). This suggests that despite fairly restrictive dietary regimen was adopted, fertility and estrus cycling was not disrupted by eTRF.

To evaluate the effect of gestational eTRF on reproductive outcomes that are similarly observed and often impacted by gestational food restriction, we calculated 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 (**Figure 4C**, p=0.20). There was a 28% reduction in the number of pups surviving to PND3 in eTRF litters (**Figure 4D**, p=0.039). Litter sizes were 15.3% smaller in eTRF dams: though this did not reach statistical significance (**Figure 4E**, p=0.072). Despite smaller litter sizes in eTRF dams, the average weight of each pup was similar between maternal dietary treatments (**Figure 4F**, 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. We suspect that reduced survival may be due to maternal cannibalization, which is common in mice undergoing nutrient restriction. We suspect this because litters were monitored daily, and the majority of the pup loss occurred within 48 hours of discontinuation of the eTRF regimen. As stated previously, it is evident that transitioning onto eTRF takes a number of days for animals to anticipate this feeding pattern and compensate with appropriate calorie intake. We therefore think it is likely that dams upon giving birth were anticipating continued restriction, and cannibalized pups more frequently than dams that were fed AL and did not experience restriction during pregnancy.

## Pup growth to PND 21 is unchanged in offspring of eTRF dams

To assess if there was an early postnatal effect of gestational eTRF on pup body weights, we weighed pups at birth, and on postnatal days 3, 7, 14, and 21. Then, using linear mixed effect modeling with random effects of pup and maternal id and fixed effects of postnatal age, pup sex, and maternal dietary regiment, we found no differences in body weight in the first 21 days of life (**Figure 4G**, p=0.073). This suggests that despite the restrictive nature of this dietary exposure, there was no evidence of growth restriction during early life in either male or female pups.

# Discussion

To our knowledge, this is the first report of the effects of 6-hour eTRF on maternal food intake and insulin sensitivity. We find that despite the very narrow window of food availability, there are negligible effects on the dam. There is no evidence of reduced weight gain, calorie restriction, or fasting glucose values in dams who are exposed to eTRF. The effect on offspring also appears to be mild, but difficult to translate to humans; smaller litters and reduced pup survival rates. The more comparable indices of gestational age and birthweight remained unchanged in eTRF dams. This study contributes to our understanding of the implications of eTRF during pregnancy on gestating parents as previous studies, namely in rats, that evaluated time restricted feeding either exclude findings in the dam (Upadhyay et al., 2019, 2020) or find significant reduction in food intake and more modest gains in body weight during pregnancy (Alkhalefah et al., 2021b). However, the latter work was meant to model Ramadan fasting, and as such, food intake was outside of the nocturnal eating window in rodents. So results must be interpreted carefully, as they are also in the presence of chronodisruption during pregnancy, which is thought to cause adverse fetal outcomes (Salazar et al., 2018) including increased risk of miscarriage (Begtrup et al., 2019). Despite insulin resistance of pregnancy being present, we found that eTRF during the perinatal period in dams resulted in no improvements in insulin sensitivity. We did find that there was more robust recovery in blood glucose after hyperglycemia in eTRF dams, which may suggest that there could be more gluconeogenesis and glycogenolysis in these dams. However, we were not able to evaluate this in the current study. We did not find evidence of reduced fasting blood glucose from gestational eTRF, which is in line with studies in humans that find no differences in glycemia (Hutchison et al., 2019; Jamshed et al., 2019; Sutton et al., 2018).

The similar pup weights in eTRF dams and continued normal body weights in both sexes after birth is in opposition to other studies where either male offspring of TRF dams weights at birth are reduced (Prates et al., 2022), or male and female fetal weights are smaller than AL counterparts (Alkhalefah et al., 2021b). Studies that find reduction in birth weights also find that dams are calorie restricted during pregnancy, resulting in reduced maternal weight gain, which could explain the lack of this phenotype in our current work.

Although the sample size for our estrus data is limited to one cohort, we did find that there were no impacts on estrus cyclicity. One study that more rigorously evaluated fertility in response to TRF found it resulted in greater follicle counts, and increased number of estrus cycles compared to AL, in both chow and HFD females(Hua et al., 2020). Although this was this in the context of pre-conceptional dietary changes (Hua et al., 2020). Where we found reductions in litter sizes Hua and colleagues litter sizes were increased in high-fat diet fed dams who had previously been pregnant (Hua et al., 2020). Results from the current study must be interpreted with caution, as latency to plug and estrus staging are less robust assessments of fertility than is ovarian sectioning, continued monitoring of breeding success rates, and counting of ovarian follicles or corpus lutea. Of note, we are the first to report reduced survival rates in eTRF offspring. We suspect this may be related to cannibalization, which is common in the strain we used in the study (Brajon et al., 2021). Dams who were fed eTRF were likely anticipating continued food restriction after birth, as is evident about the adaptation period stated previously. Moreover, the reduction of survival in pups born in large litters is difficult to translate to human pregnancy.

Preclinical models are an imperfect proxy for human pregnancy for many reasons, but the evidence from this study may have translational value in pregnant human populations. Given the lack of growth restriction in offspring in early life and absent maternal weight loss, nutrient restriction, and insulin dysmetabolism, this may warrant further evaluation in pregnant humans in controlled spaces. Other outcomes such as latency to plug, 3-day pup survival, and litter sizes are not as easily translated to human populations. However, as the majority of the deleterious effects that arise from this dietary treatment in the current study are difficult to translate, it would be presumptuous to say that negative effects are unlikely in human pregnancy.

As with any experimental model, this work has some limitations. One such limitation is the lack of molecular mechanisms investigated at the level of both dam and pup. We sought to evaluate the phenotype at a basic level in this study and as such were not able to investigate changes at the tissue level in either dams or pups. It is possible that despite the lack of overt metabolic differences in dams and body weights in pups that there were more nuanced differences in metabolically active tissues or even within the central circadian clock. Future work should devote attention to analyses. Another limitation is that fertility outcomes were only cursory in assessment, and in a reduced number of dams. As the effect of intermittent energy restriction on fertility and reproductive health is a concern in non-pregnant females, more stringent study and in greater numbers should be devoted to this question.

The current study has many strengths. First, the design was carefully considered to ensure handling stress was minimized and the timing of eating was in line with natural mouse rhythms. As such, results can be separated from effects from chronodisruption. This was repeated in 3 separate cohorts resulting in large samples sizes for both dams and pups. This suggests, the observed phenotype is likely to be reproducible with other groups using a similar paradigm.

# Conclusion

In summary, we find that eTRF feeding of dams during the perinatal period results in very few changes in the physiology of the dam, only a greater rebound in glucose after insulin challenge. There is similar rates of pregnancy and fecundity in dams fed eTRF. We find that pups born to eTRF dams are of similar size and grow in comparable ways to AL offspring. The deleterious effects noted are a reduction in litter size and in pup survival to postnatal day 3, although the reason for these reductions in not clear. Further work must be done to scrutinize the safety of this practice and efficacy for ameliorating metabolic illness during pregnancy in higher risk populations.

# Figure Legends

*Figure 1:Early Time-Restricted Feeding in a Mouse Pregnancy Model*

A) Food availability and light:dark cycle for early time restricted (eTRF) and *Ad Libitum* (AL) dams. Beginning one week before mating and lasting through parturition (eTRF n=16, AL n=14), eTRF dams had food access from chow access between ZT14-ZT20, AL dams had 24-hour chow access. B) Maternal dietary treatment differed in the perinatal period. On E16.5, an intraperitoneal insulin tolerance test was conducted. Blood samples were collected at ZT1 and ZT13 the day after ITT under normal feeding conditions for each dietary regimen. All dams were maintained on chow AL after birth. Pups were reduced to 4 per dam on PND 3. Pups were weighed at birth, PND 3, 7, 14, and 21.

*Figure 2: eTRF in Dams does not affect 24-hour Food Intake or Body Weight During Pregnancy*

A) The adaptation period to eTRF from first day of exposure until day 7 of experiment. Dams randomized to eTRF slower increased food intake in the 6-hour window from AL levels. B) Six-hour food intake (between ZT14-ZT20) in the week before copulatory plug appeared. C) Food intake from ZT14-ZT20 one week before copulatory plug appeared. C) Food intake from ZT14-ZT20 from gestational day 0 until birth. D) Food intake over 24 hours (ZT14-ZT14) one week before copulatory plug appeared. E) Food intake over 24 hours (ZT14-ZT14) during pregnancy (E0-birth). F) Body weights (grams) in dams one week before copulatory plug appeared. G) Body weight in dams (grams) from E0-birth. \*Indicates p-value of diet <0.05. If p-value is not for diet alone, it is labeled with interaction. (eTRF n=16, AL n=14)

*Figure 3: Rebound after Hypoglycemia is increased in eTRF Fed Dams During Late Gestation*

A) Intraperitoneal Insulin Tolerance Test (ITT) in dams on E16.5 after fasting 6 hours (ZT2-ZT8). B) Fasting blood glucose (mg/dL) before insulin administration. C) Area Under the Curve of ITT. D)Rate of glucose decline from modeling exponential rate of drop in glucose from time 0 to 45 minutes. E) Rate of linear increase in glucose from 60-120 minutes during ITT. F) Serum insulin concentration at ZT1 and ZT13 in dams the day after ITT. \*Indicates p-value of diet <0.05. If p-value is not for diet alone, it is labeled with interaction. (eTRF n=11, AL n=11)

*Figure 4: eTRF Does not Affect Fertility, but Reduces Pup Postnatal Survival and Litter Size*

A) Total days spent in each stage of the estrus cycle before initiation of mating in dams (eTRF n=7, AL = 6). B) Time in days from pairing of male and female to copulatory plug appearance. C) Total days from copulatory plug appearance to birth of litter D) Percent of pups per litter who survived from birth until postnatal day 3. E) Number of pups per dam. F) Average weight of pup per litter on PND 0 (grams). G) Body weight of pups by maternal dietary treatment and pup sex (Male eTRF n=23, Male AL n= 33, Female eTRF n= 34, Female AL n= 27, eTRF pups culled before sexing at PND 3 n=53, AL pups culled before sexing at PND 3 n=40). Sample numbers for latency to plug, gestational age, 3-day survival, litter size, and birthweight are the same ( eTRF n=16, AL n=14).

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