**Translational Aim 3: Characterize the prevalence and associations of restricted feeding with maternal and child health in humans.**

I will first characterize the prevalence of pregnancy-associated complications in the biorepository for understanding maternal and pediatric health (BUMP) cohort, then will further investigate the associations of the length of feeding window with perinatal health outcomes including: preterm birth, small for gestational age, intrauterine fetal demise, gestational diabetes, gestational weight gain, and pre-eclampsia.

**Background:**

Fasting in pregnancy

Intermittent fasting during pregnancy has not been thoroughly examined in humans. The closest analogue to the TRF paradigm of IF would be fasting in Ramadan. [Insert Ramadan graphic here]. In general, Ramadan fasting is not a good mimetic for the TRF paradigm of IF, as it often is accompanied by changes in sleep patterns and food quality, both of which could independently affect disease risk and health.

Neonatal Health Outcomes

Preterm birth is a significant health risk for neonates. It has been demonstrated that infants born before term (37 weeks’ gestation), are at greater lifetime risks for higher total cholesterol, triglycerides, glucose and insulin as well as high blood pressure (Suzuki, 2018)(Lewandowski Adam J. et al., 2015). Because pregnancy is a complex period of rapid adaptation for the mother, the etiological drivers of pre-term birth have been difficult to isolate and study. Mothers with short stature, lower educational attainment, who smoke, or have diabetes are more likely to deliver before term (Kramer, McLean, Eason, & Usher, 1992). Some of these risk factors can directly be tied to nutritional status, such as diabetes. However, many cannot be directly corrected by nutrition, but would likely have consequences for maternal nutritional status, such as increased need for water soluble vitamins in those who smoke, lower fruit and vegetable intakes in those with lower incomes, and lower food security for women who have lower educational attainment. This can be difficult to disentangle from other conditions that are associated with pre-term birth, such as infant birth weight.

SGA/low birthweight

Infant birth weight is associated with increased risk of death before one year of age, increased risk for obesity and chronic disease, and \_\_\_\_.

Maternal Health Outcomes

Gestational weight gain

The appropriate amount of weight that is to be gained for a healthful pregnancy is drawing attention from both clinicians and researchers in recent years, and recommendations have been tailored to pre-pregnancy BMI to optimize offspring health outcomes (INSERT REPORT). Gestational weight gain has been associated with offspring body mass index and risk of obesity from infancy all the way through adulthood (Schack-Nielsen, Michaelsen, Gamborg, Mortensen, & Sørensen, 2010) .

Preeclampsia

Preeclampsia is a maternal condition of pregnancy that is characterized by high blood pressure and protein in the urine (proteinuria) and occurs after 20 weeks gestation (Chaiworapongsa, Chaemsaithong, Yeo, & Romero, 2014).

Gestational diabetes

**Translational Aim 3.1: Examine the baseline characteristics of the BUMP cohort**

Because no previous study has utilized BUMP cohort data, there must be some descriptive statistics done in order to understand what confounding variables and collinearities exist in the cohort.   
Ethical Approval, Data Acquisition, and Data management:

The recruitment of participants and collection of medical information and biospecimens has been approved by the University of Michigan Institutional Review Board (HUM00118179). I will prepare, submit, and be approved for a secondary use permit before beginning any analysis described below. I will submit requests for the full medical information I seek from charts and will be supplied with de-identified participant data. This data will be exclusively held on a secure server, with access only to those who are part of the study team. Participant data will not be downloaded on personal computers, and will be backed up on MBOX. As biological assays are conducted on biorepository tissues, they will be merged with the original dataset to maintain a single, de-identified dataset available for statistical analysis.

Study Population:

In brief, recruitment is done in the Vonn Voigtlander Women’s clinic, with special focus on the maternal and fetal medicine clinic days, who serve high risk obstetric patients. As of August 2019, this sample consists of roughly 800 women enrolled at different stages in their pregnancies. Eligible women are those who are 18 years or older, who van read and understand the consent form in English, and receive their prenatal care at the VVWH and plan to deliver at VVWH. Research assistants are told by physicians during prenatal care visits if patients are interested in enrolling in the BUMP study. The study is explained, a pamphlet is given, and if a patient is interested, the research assistant obtains written informed consent and gives the participant the questionnaire seen in appendix 1.

Inclusion criteria will be women with live, singleton births who completed at least 2 of the 3 collections during pregnancy. Pregnancies complicated by fetal anomaly, congenital birth defects, or poor placentation/placental defects, or multiple gestation will be excluded. This will result in a population of women that could be quite heterogeneous; who may or may not have obesity, may or may not have experienced gestational hypertension, gestational diabetes, preterm birth, cesarean delivery, or taken glucocorticoid drugs during the course of their pregnancy.

Collection of Biological Samples  
By participating in the study, women consent to collection of urine, blood, placenta, and cord blood. During each trimester, women who consent to be part of the study are given urine containers and asked to provide up to 100 mL of urine. Urine is then frozen and kept at the Michigan Medicine Central Biorepository under a unique study ID. Blood samples that are drawn for research purposes are coordinated to occur at the same time as prenatal lab draws to minimize participant burden. Present in the research kit are vacutainers for blood draw, which usually takes place at a Michigan Medicine laboratory. Trained phlebotomists collect 40 mL of whole blood each trimester. Blood samples are then picked up by research assistants, aliquoted, and stored Because blood samples are in coordination with prenatal labs, there may be inconsistency in fasting state of these samples. The mid-gestation blood draw is usually done in combination with the oral glucose tolerance test screen for gestational diabetes, which is recommended to occur between 24 and 28 weeks gestation (Randel, 2014). Therefore, indices like insulin will need to be interpreted with caution. After delivery and cutting of the umbilical cord, cord blood will be collected by labor and delivery nurses for clinical and research purposes, up to 40 mL of which can be used for research purposes. Upon delivery of both the infant and the placenta, a labor and delivery nurse will collect two (each sized 1x1x3 cm) placental samples. One sample will be stored in RNAlater, and another will be fixed and embedded in paraffin for histological analysis. All biological samples are stored in the Michigan Medicine Central Biorepository under a unique study ID/barcode. Study samples will not be stored with identifying information.

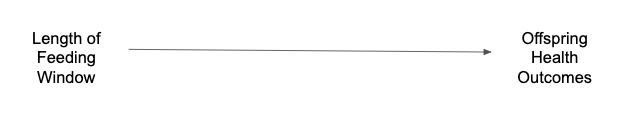
Medical Chart Data

Medical chart data is accessible to the research team and can be compiled at the request of the secondary use IRB protocol. This process allows this to access diagnosis codes for the pregnancy and child health in the subsequent infant’s chart. The proposed medical data to be retrieved from the medical chart will include: last measured pre-pregnancy height and weight, maternal age at conception, last measured gestational weight and height, maternal medication use (glucocorticoid, insulin, metformin, progesterone, aspirin, statins, ADHD medications), maternal smoking and drug use history, pre-existing diabetes or hypertension, gestational diabetes, hyperemesis gravidarum, hypertensive disorders of pregnancy, intrauterine fetal demise, birth weight of offspring in grams, gestational age at birth, and APGAR score. These medical data will then be compiled for each participant and added to the demographic and feeding window data compiled from the intake questionnaire.

Assessment of Feeding Window

In order to ascertain the window within each participant consumes their meals each day, the following questions were added to the intake questionnaire, “On a typical day during your pregnancy, when was the first time in the day you had something to eat?” to determine the beginning of the feeding period, and “On a typical day during your pregnancy, when was the last time you had something to eat before going to bed?” to assess the closing of the feeding period. Ideally, the responses will be grouped into a categorical variable consisting of 2-hour differences in length of the feeding window:   
>12 hours, 10-12 hours, 8-10 hours, 6-8 hours, and <6 hours. These feeding windows are all reflected in the TRF literature (Rothschild, Hoddy, Jambazian, & Varady, 2014).

**Translational Aim 3.2. Investigate the associations of feeding window length on maternal and child health outcomes**

Figure XX:

No studies to date have evaluated the effects of time-restricted feeding on the incidence of maternal and child outcomes in human populations. There have been studies on the effects of Ramadan fasting during pregnancy on these outcomes. As stated previously, Ramadan fasting is inconsistent in its findings. Some studies associated fasting with lower birth weight, whereas others see no effect on child age. Studies on gestational age are somewhat more consistent in that there is no apparent relationship between Ramadan fasting and preterm birth. Although imperfect, one such cross-sectional study evaluated a consistent exposure to Ramadan fasting and categorized participants based on the level of fasting completed (1-10d, 11-20d, 21-29 days in the 2017 month of Ramadan). They found that Ramadan fasting only affected two of their nine outcomes. The odds of gestational diabetes were lower in expectant mothers who participated in the Ramadan fast (2.6% vs 8.3%)(Safari, Piro, & Ahmad, 2019). The strength of the association remained after adjusting for maternal education, maternal age, maternal occupation, parity, and pre-pregnancy BMI (OR = 1.51 (0.06+/-1.74)). There were no observed differences in rates of preterm labor, preeclampsia, low birth weight, fetal height, fetal head circumference, or APGAR score at 5 minutes . Based on the work done during Ramadan fasting in pregnancy and other TRF literature, *I anticipate that women who have shorter eating windows are less likely to develop pregnancy related maternal health issues (diabetes, preeclampsia, hyperemesis gravidarum).*

To assess the associations of feeding window length with maternal and child health outcomes, multiple linear regression analysis will be used for the categorical and continuous outcomes; such as offspring birth weight, gestational age, and APGAR score. For dichotomous outcomes, such as gestational diabetes, hypertensive disorders of pregnancy, and hyperemesis gravidarum logistic regression will be employed.

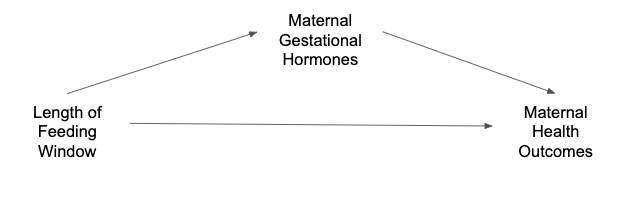
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Figure X: Depicts the DAG for aim 3.3

**Translational Aim 3.3 Examine the molecular basis for feeding window association with maternal and child health in biological samples**

The metabolic and biochemical effects of fasting during pregnancy are critically understudied. It is known that a large proportion of the Islamic world chooses to fast when pregnancy, for many reasons; among them\_\_\_\_\_\_\_ (Safari et al., 2019). However, as pregnant mothers are a particularly difficult group to enroll and study, there is very little data on what fasting does to maternal and child hormonal and nutritional signals. Of those few studies, the majority exist as case studies or observational studies of women with existing diabetes during pregnancy (CITE). Observational studies of pregnant, normo-glycemic women who observed Ramadan fast noticed that women had higher post-prandial glucose responses after breaking the fast than pregnant controls (measured one hour after first meal in the morning) (Baynouna Al Ketbi, Niglekerke, Zein Al Deen, & Mirghani, 2014). This sample is imperfect in a few ways. The two samples were taken at different times of day, which is known to affect glycemia and insulinemia in humans. Furthermore, the measurement of fasting and post-prandial glucose alone is insufficient to begin to understand the mechanisms that may be activated by maternal fasting. A baseline understanding of the associations of habitual dietary fasting and markers of metabolic health in the blood of pregnant women is a critical need. The BUMP cohort biological samples (blood, and urine) can help to fill that gap. The hormones to be assayed in the blood samples is largely to be determined by the results of the model organism aims of this proposed dissertation work. The initial list will be comprised of blood insulin, glucose, GDF15, and cortisol provide the strongest candidates. Each will require 50uL of plasma, totaling 200uL minimum of each sample.

To provide translatability to the animal study, human maternal blood samples will be evaluated for insulin and glucose content. Further analysis for other contributing hormones are to be added based on evidence from the animal cohort.

**Methods:**

*Human blood hormone determination: ELISA*

Human blood insulin concentration will be determined by ELISA (crystal chem catalog #90095). This highly reactive and non-cross reactive assay for C-peptide will be done in duplicate for each sample. Briefly, 25uL of plasma from each sample will be diluted with 100uL of diluent (from the kit) in a well of a 96 well plate (Axygen, Corning Catalog # PCR- 96-FLT-C) and incubated for 2 hours at 37 degrees C. Samples will then be washed, and 100uL substrate solution will be added to each well, then left to incubate for 15 minutes at 25 degrees C. Plate will then be washed and 100uL of stop solution will be added. Plate absorbance will then be read at 450/630 nm on (make and model of plate reader in Seo lab). Insulin concentration will be calculated using a standard curve fit to known concentrations of a set solution. Individual observations will be reported as the mean concentration (pg/mL) of the two replicates.

*Statistical Analysis*

Statistical analysis will be conducted in R (version).

Univariate and Bivariate Analyses

In order to assess the distributions of the maternal feeding window data and gestational and infant outcomes, I will conduct a univariate analysis of each variable independently. This will determine whether or not these variables need to be normalized for use in multiple regression and will determine cutoffs for the categorical variables. Bivariate analyses will then be conducted by examining the distributions of the outcome variables across socio-demographic and maternal health indices. This will identify variables that should be considered confounders in the relationship between the feeding window during pregnancy and maternal and child health outcomes.

Multivariate Analyses

To assess the associations of feeding window length with maternal and child health outcomes, multiple linear regression analysis will be used for the categorical and continuous outcomes; such as offspring birth weight, gestational age, and APGAR score. For dichotomous outcomes, such as gestational diabetes, hypertensive disorders of pregnancy, and hyperemesis gravidarum logistic regression will be employed.

*Proposed models:*

Logistic models:

Model 1: eating window = maternal health outcomes

Model 2: Model 1 + Maternal education  
Model 3: Model 2 + Gestational Weight Gain

*Predicted confounders for bivariate analysis:*

Maternal gest age at enrollment  
Maternal race/ethnicity  
Household Income  
pre-pregnancy BMI  
Gestational Weight Gain

Smoking Status

Sleep quality

Offspring Sex

**Potential Pitfalls and alternative approaches:**

*Low recruitment/underpowered in the feeding windows*

Because recruitment is part of an observational instead of interventional study, we will not know the length of the feeding window until after the recruitment process. Because of this, there may not be an even distribution of women in each of the designated feeding window groups. If this is the case, there are two alternative approaches. The first is to continue recruitment and the second is to analyze the distributions of the feeding window variable collected and to use more iterative cut offs that are apparent in the data.

*Lower or unrepresentative incidence of disease states*

As this study proposes to use participant data that is recruited in an untargeted manner, it is possible that recruitment could result in lower than expected incidence of the maternal outcomes investigated. It is also possible that because of the prestige in neonatal care associated with this hospital could attract a greater than expected incidence in maternal health outcomes. Either situation could affect the generalizability of the results of this study.

*Confounding of feeding variable by dietary quality*

While the purpose of this proposed dissertation work is to further the understanding of the relationship between length of time fasting and maternal and child health, using only the length of the fast as the only dietary measure is a limitation. There are many components to consider when attempting to understand dietary adequacy, and timing of meals is only one. Others such as dietary quality, macro- and micronutrient adequacy, macronutrient distribution, energy intake, and food safety are all concerns that may affect the relationship. Because dietary quality data is not collected for this sample, we cannot say that associations determined are exclusively because of the feeding window. However, the literature on TRF has shown some robust effects in models that utilize Western, high-fat dietary exposures, meaning there could still be some relationship of the feeding window to outcomes independent of diet quality and calorie intake.

*Poor reliability of fasting state in blood samples*

The BUMP cohort design makes it reasonable and easy for participants to enroll in the study, and one fantastic example is in the drawing of neonatal labs. It may be unreliable to assume a similar level of feeding or fasting in these samples. For that reason, insulin and glycemic health data sensitive to fasting/refeeding cycles will be interpreted with caution. It may be of benefit to specifically choose the mid-gestation collection point, as this is done in concert with the oral glucose tolerance test, and therefore is likely to be more uniform in the feeding level (75g of glucose within 1 hour of blood draw).

*Residual Confounding*

As is the case with any observational study, the inclusion of any confounding variables is a best attempt at reducing the relationship between the outcome and the exposure through the causal pathway, but there is also potential for residual confounding. Furthermore, as the intake questionnaire is both quite simplistic and could simply not measure a confounding variable that could occlude the relationships we are looking for.

Appendix 1: BUMP Study Intake Questionnaire  
  
Page 1: University of Michigan Pregnancy Biorepository Study ID: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

HUM00118179 Date: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

1. **What is your Ethnicity:**

⃞ Hispanic or Latino or Spanish Origin

⃞ Not Hispanic or Latino or Spanish Origin

⃞ Unknown

⃞ Prefer not to say

1. **What is your Race (check all that apply):**

⃞ American Indian or Alaska Native

⃞ Asian

⃞ Black or African American

⃞ Native Hawaiian or Other Pacific Islander

⃞ White

⃞ Unknown

⃞ Prefer not to say

⃞ Other 🡪 Please describe: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

1. **What is the highest level of education you have completed:**

⃞ Some high school, no diploma

⃞ High school graduate, diploma or equivalent (for example: GED)

⃞ Some college credit, no degree

⃞ Trade/technical/vocational training

⃞ Associate degree

⃞ Bachelor’s degree

⃞ Master’s degree

⃞ Doctorate or Professional degree

⃞ Prefer not to say

1. **What is your annual household income:**

⃞ $11,999 or less

⃞ $12,000 to $24,999

⃞ $25,000 to $49,999

⃞ $50,000 to $99,999

⃞ $100,000 to $149,999

⃞ $150,000 or more

⃞ Prefer not to say

1. **How would you best describe your marital or partnership status:**

⃞ Single, never married

⃞ Married or domestic partnership

⃞ Widowed

⃞ Divorced

⃞ Separated

⃞ Prefer not to say

⃞ Other 🡪 Please describe: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Page 2: University of Michigan Pregnancy Biorepository Study ID: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

HUM00118179 Date: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

1. **How many people are in your household (including yourself):** \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
2. **Do you currently snore 3 or more nights a week?**  ⃞ Yes ⃞ No ⃞ I don’t know
3. **Before your pregnancy did you snore more than 3 nights a week?**  ⃞ Yes ⃞ No ⃞ I don’t know
4. **Are you currently a smoker?**  ⃞ Yes ⃞ No

9a. If yes, how much do you smoke per day? \_\_\_\_\_\_\_\_\_

1. **Are you a former smoker?**  ⃞ Yes ⃞ No

10a. If yes, when did you quit? \_\_\_\_\_\_\_\_\_\_

1. **Are you regularly exposed (several times/week) to someone else’s smoke during the past 3 months?**

⃞ Yes ⃞ No

1. **Do you live near a landfill (less than 2 miles)?**  ⃞ Yes ⃞ No
2. **Please let us know if you use any of the following personal care products on a regular basis:**

Perfumes and cosmetics  ⃞ Yes ⃞ No

Hair care products ⃞ Yes ⃞ No

1. **Have you had dental fillings in the past 3 months?**  ⃞ Yes ⃞ No
2. **Do you eat canned foods (at least once a week)?**  ⃞ Yes ⃞ No

**15a. If yes, how often do you eat canned food?**

⃞ 1 serving or less/day ⃞ 2-3 servings a day ⃞ 4 servings or more/day

1. **Do you eat at fast food restaurants (at least once a week)?**  ⃞ Yes ⃞ No

16a. **If yes, how often?**

⃞ once a week ⃞ 2-3 times/week ⃞ 4 times or more/week

1. **Do you eat fresh vegetables (at least once a week)?**  ⃞ Yes ⃞ No

17a. **If yes, how often?**

⃞ 1-3 servings/day ⃞ 4-5 servings/day ⃞ 6 or more servings/day

1. **Do you feel stressed?**  ⃞ Yes ⃞ No

**18a. If yes, how often do you feel stressed?**

⃞ Never ⃞ Almost Never ⃞ Some Days ⃞ Most Days ⃞ Every Day

**Works Consulted**

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