THE ASSOCIATION BETWEEN MATERNAL DIET, GESTATIONAL WEIGHT GAIN, AND NEONATAL BODY COMPOSITION

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Abstract

Obesity is an ever-growing concern in the United States and throughout much of the world. While our understanding of what impacts obesity and related health concerns, there is a growing body of evidence that suggests that programming that occurs *in utero* may play a significant role in the development of obesity and other, related disease such as diabetes and cardiovascular disease. Maternal diet during pregnancy remains and area that has yet to be fully described in terms of the relationship to important predictors of long-term health, including maternal weight gain during pregnancy and infant adiposity at the time of delivery. Our study enrolled 41 women receiving prenatal care for singleton, uncomplicated pregnancies at Oregon Health and Science University in Portland, Oregon. Maternal diet was calculated by averaging three 24-hour food recall questionnaires collected during their third trimester of pregnancy. Linear and logistic regression modeling was done to evaluate the association between maternal diet and three outcomes: gestational weight gain (GWG), adherence to GWG recommendations and infant adiposity. The results of our study demonstrate the maternal diet in late pregnancy is not associated with GWG, or with adherence to GWG recommendations. We also demonstrate that maternal diet composition in late pregnancy is not associated with infant adiposity at the time of delivery. These findings are significant in that they suggest that our approach to GWG and infant outcomes should be focused on providing appropriate guidance and support for women prior to conception rather than during gestation.

Section 1. Introduction

During pregnancy increased maternal fat stores, decreased maternal insulin sensitivity, other metabolic adaptations occur to allow for essential nutrient delivery to the fetus during its growth *in utero.* [1,2] These metabolic shifts lead to increased protein[3,4] and caloric needs[5,6] as well as various micronutrient requirements.[7] However, there is currently a lack of evidence-based guidelines for diet composition during pregnancy. Clinical medicine and public health campaigns continue to rely on broad generalizations about healthy eating and balanced diets[8–11] during this crucial life stage, often drawing on what is known about the non-pregnant population. This represents a problem because the physiologic changes that take place during pregnancy limit the generalizability of our knowledge from the non-pregnant to pregnant populations.

Furthermore, growing evidence suggests that the prenatal period is critical in the development of disease in the next generation[12,13] and long-term health for the mother. Risk of heart disease, diabetes and obesity is greater in infants born to mothers with excessive gestational weight gain (GWG),[14–16] or who have elevated adipose tissue at birth, often approximated by birth weight.[17–21] In addition to the risks posed to the long-term health of their offspring, gaining weight above what is recommended during pregnancy poses long-term health risks to mothers, including postpartum weight retention and elevated risk of obesity.[22–24] Collectively, these issues reflect a need for research on the association between maternal diet in pregnancy and maternal weight gain and infant adiposity, two important predictors of long-term health in these populations.

Potential health impacts of maternal diet composition

To help fill this pressing evidence gap, we used a framework positing a connection between maternal diet composition on the long-term health of children and mothers.

Within this framework, we focused on three health outcomes: gestational weight gain as a continuous outcome, gestational weight gain categorized based on adherence to the 2009 IOM guidelines for recommended weight gain, and infant adiposity at the time of delivery.

First, weight gain is essential to most normal pregnancies, allowing for fetal growth and development.[2,25] The weight gained during this period is influenced by numerous factors including parity, smoking history, pre-pregnancy Body Mass Index (BMI, kg/m^2)[26,27] and sociodemographic characteristics, including prenatal smoking, young maternal age and low income.[28,29] Several observational studies suggest that diet and physical activity may play an important role in gestational weight gain (GWG),[8,11,30] however a deeper analysis of the relationship between macronutrient intake and GWG has yet to be described. The association between macronutrient intake (dietary intake of fats, carbohydrates and protein) and the association with GWG varies across studies, some showing a greater probability of excess GWG with greater intake of fats and protein[31] or consumption of a diet with a higher glycemic index or foods with greater energy density.[29,32] While some inconsistencies in the body of literature on this topic may be the result of different approaches to studying this topic (e.g., different exposure and outcome measures, different definition of GWG used) there is also a dearth of high-quality evidence in this area. Many studies used poor dietary measures to estimate macronutrient intake, had small sample sizes, or inadequately controlled for confounding in their analysis.[33] The lack of consistent findings to date suggests there is much to be learned in this area.

Second, GWG in excess of recommendation is associated with adverse outcomes including increased risk of cesarean section, postpartum weight retention, increased risk of gestational diabetes (GDM) and large for gestational age (LGA) infants. [34–37] The adverse outcomes associated with excess weight gain during pregnancy make it an important indicator of risk for poor long-term health of mothers. Given the risk associated with

gaining in excess of recommendations, it is important to understand what role, if any, diet plays in this outcome.

Third, greater adiposity at the time of birth is an indicator of higher risk for obesity,[15,18,19] cardiovascular disease, and diabetes[23,38–40] later in life. Animal and epidemiologic research has demonstrated an important association between maternal diet, in terms of caloric needs, and long-term health effects of offspring,[41] with the majority of human evidence based on regions afflicted by famine. However, little is understood about the impact of diet composition on neonatal adiposity in humans. Birth weight and ponderal index (PI), which are established, measurable, predictors of outcomes such as obesity and diabetes,[15,42] and have traditionally been used as an approximation of infant adiposity. However, the high variability of normal birth weights among term infants limits its use as an indicator of the intrauterine environment and development of the fetus.[17] This variability is the result of numerous factors such as gestational age, genetics, and environmental drivers.[43,44] An alternative to birth weight, PI, has also been used, however this measure explains only 22% of the variance in birth weight, compared to skin fold thickness measurement (SFTM) of infant adiposity, which explain 46% of this variance, demonstrating the advantage of using SFTM or other measures of infant adiposity over birth weight and PI. [45]

Objectives

Beyond meeting caloric and nutrient needs of mother and baby, macronutrient composition may play a significant role in the development of the fetus and the imprinting that takes place *in utero,*[46] predisposing newborns to lifelong metabolic abnormalities and mothers to adverse long-term health outcomes. Our study aims to improve our understanding of the impact of diet during pregnancy on GWG and neonatal adiposity,

allowing for a better appreciation of how this modifiable risk factor relates to the long-term health of mothers and infants as well as the development of evidence-based, individualized prenatal counseling and support. In particular, although GWG is often assumed to be a modifiable exposure (and therefore an appealing target of clinical and public health intervention), little research documents the existence and potential mechanisms of this association. Therefore, our objectives were to estimate the effects of maternal macronutrient intake in the third trimester on three outcomes: gestational weight gain as a continuous outcome, gestational weight gain categorized based on the 2009 IOM guidelines for recommended weight gain, and infant adiposity at the time of delivery, an important and under-studied intergenerational outcome. By conducting this research, we aimed to more fully explore the relationship of diet, a potentially modifiable risk factor on GWG and infant adiposity in hopes of developing more evidence-based, targeted guidelines to educate women during pregnancy.

Section 2. Methods

Study Population

Study Overview and Data Collection. This study is a secondary data analysis of data from the NIH-funded Maternal Body Composition (MBC) study (PI: Dr. Nicole Marshall). The MBC was a prospective, observational cohort study designed as a pilot study to assess the role of maternal body composition on maternal and neonatal outcomes. All women enrolled received standard of care gestational weight gain and dietary counseling, per recommendations by the American College of Obstetricians and Gynecologists[47] and the Institute of Medicine.[48,49]

Subjects. All women enrolled in the MBC study were eligible for inclusion in this secondary data analysis. Women were eligible for the MBC study if they were non-diabetic with no evidence or history of chronic disease and were pregnant with an uncomplicated, singleton pregnancy, at term (greater than or equal to 37 weeks' gestation) and receiving prenatal care at OHSU. A pregnancy was considered uncomplicated if there were no documented fetal anomalies on ultrasound, if the woman had no history of abnormal glucose tolerance test during pregnancy and had no diagnosis of gestational diabetes. All women receiving care from any obstetric provider at OHSU, including family medicine physicians and certified nurse midwives, were eligible for enrollment. Pre-screening of women took place using electronic medical record (EMR) data to determine women who would likely meet inclusion criteria with regards to timing in their pregnancy, singleton pregnancy and without gestational diabetes. A pre-screening letter was sent to women identified by EMR, inviting them to participate in the study if interested.

Women were screened via the inclusion/exclusion criteria questionnaire to determine their eligibility, Table A, Appendix A. Once women were determined to be eligible for the study they were asked to complete a social and medical history, Table B in Appendix A, which was completed during Study Visit One, see timeline, Figure 1, below.

Participants in the MBC study attended two study visits, during which interviews and clinical measurements were performed, in addition to infant measurements taken around the time of delivery (See Figure 1). Visit 1 (at approximately 37 weeks) included an antepartum maternal body composition assessment. Women were also asked to complete both a Block Food Frequency Questionnaire (FFQ) and one 24-hourFood Recall during this visit. Two additional 24-hr food recall questionnaires were collected via phone, by trained study staff, between visit 1 and 2. Weight was measured at the final prenatal visit in the week prior to delivery, to determine GWG. Within 24 hours following delivery, infants were

measured, including length, weight, abdominal circumference and skin-fold thickness. Visit three took place two weeks following delivery, during which repeat maternal body composition measurements were performed, as shown in Figure 1, below.

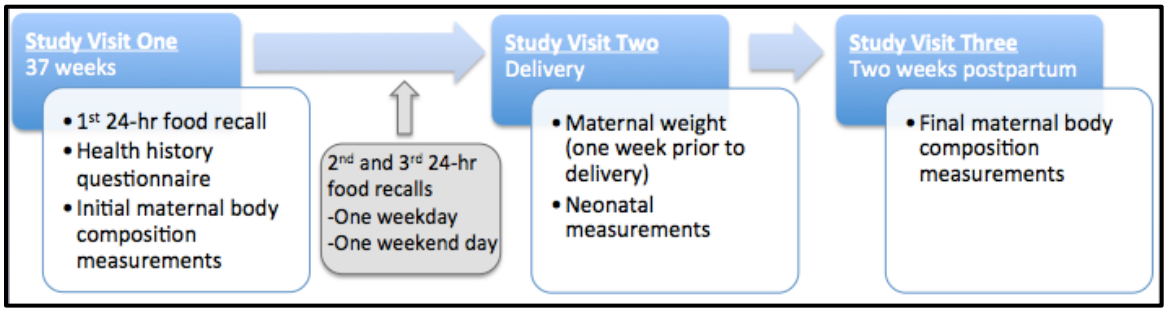

Figure 1. Study visits for women meeting inclusion criteria for MBC study.

Study Variables

Exposures. We used 24-hour dietary recall data to assess macronutrient intake in the 3rd trimester as it is a well-validated tool for estimating average diet over a short time period.[50] Three 24-hour dietary recalls were collected (Figure 1). The first was performed during visit one, the following two between the first and second study visit (at the time of delivery). The second and third 24-hour recalls took place over the phone with trained study staff and included one weekday and one weekend day. Data from the three 24-hour dietary recalls were averaged to approximate diet during the 3rd trimester of pregnancy. Using these averages, maternal macronutrient intake (fat, protein and carbohydrate) was calculated as total grams consumed and as a percent of daily caloric intake. In this study, we analyzed percent calories from each macronutrient, as the caloric density varies significantly between each macronutrient. [51]

Outcome: Gestational weight gain. Gestational weight gain (GWG) was calculated as the difference in weight prior to pregnancy (collected from EMR) and weight at last prenatal

visit (approximately one week prior to delivery). In primary analysis, GWG was evaluated as a continuous variable, controlling for pre-pregnancy BMI.

We also examined adherence to the 2009 IOM GWG guidelines,[51] which are based on pre-pregnancy BMI. Using these recommendations three categories were created: women that gained within the recommended amount of weight for their pre-pregnancy BMI, above the recommended amount or below it. Maternal pre-pregnancy BMI for each individual was calculated using measured height, obtained at first study visit, and pre-pregnancy weight (as reported in the EMR). For purposes of data exploration and classification of adherence to the Institute of Medicine (IOM) guidelines for GWG, BMI was categorized as normal (BMI$\leq$25 kg/m^2), overweight (BMI 25-29.9 kg/m^2) and obese (BMI$\geq$30 kg/m^2),[27] in accordance with WHO guidelines.[26,27]

Outcome: Neonatal adiposity. Neonatal body composition measurements were taken within twenty-four hours of delivery. Skin fold thickness measurements (SFTM) were collected by study staff, in accordance with previously documented and widely accepted procedures[52] as well as additional measurements including, weight, abdominal circumference and length.

Neonatal fat mass was calculated using the equation developed by Catalano, et al.[52] This equation, a modification of an earlier equation developed by Dauncey, et al. in 1977[53] provides a more accurate method for estimating total body fat in infants and is a stronger predictor of infant adiposity than the more commonly used Ponderal Index (PI) or birth weight. [45] While birth weight is a traditional measure of infant adiposity[14], it serves as only a rough approximation of body fat.[12] Skinfold thickness measurements in infants are rare, especially in populations where maternal dietary measures are also collected.[17] [19] A more direct measure of neonatal fat mass, such as SFTM, which may more strongly predict long-term health effects,[18] including obesity, cardiovascular disease and diabetes, compared to

birth weight or PI. Very few studies have used SFTM, to estimate infant adiposity, despite the fact that it is a better predictor of infant adiposity and long-term health outcomes, compared to PI and birth weight.

Covariates Demographic, social and medical history data were collected via questionnaire during study screening and Study Visit One. However, given small sample size, covariates included in the model were limited to those known to have clinical significance to the exposure and outcomes of interest. Maternal age (at time of delivery) was analyzed as a continuous variable. Parity was dichotomized (nulliparous versus multiparous), as only two women had more than one prior pregnancy.

Maternal pre-pregnancy BMI was retained in the models as it is a well-recognized risk factor[54] for gestational weight gain as well as for birth weight. Although categorized GWG is pre-pregnancy BMI-specific, we included continuous BMI as an independent variable in order to control for this potential confounder and allow a more clear exploration of the relationship between this variable and the outcomes.

Statistical Analysis

Descriptive and Exploratory Analysis All statistical analysis was performed using Stata/MP©, version 13.1. Distributions of continuous dependent variables were examined to assess normality, in order to meet requirements for use of linear regression modeling. Q-Q plot of residuals, box, plots and scatter plots were also used (Figure 2).

Spearman's correlation was used to evaluate crude relationships between each continuous independent variable and both continuous outcomes (GWG and infant adiposity). Correlation coefficients >0.6 were considered concerning for collinearity. ANOVA was used to assess independence of parity (dichotomous) for both continuous

outcomes, GWG and neonatal fat, as well as to assess differences of continuous independent variables across categories of GWG. Fisher's exact was used to evaluate relationships between categorized GWG and both categorical independent variables, BMI (categorized) and parity, given small cell sizes. ANOVA was used to assess whether there were significant differences for the mean of each continuous independent variable across category of GWG adherence.

Regression Analysis Linear and multinomial logistic regression analyses were used to estimate the effect of diet during the third trimester of pregnancy on three outcomes: continuous GWG, adherence to GWG recommendations (categorical) and neonatal adiposity (percent of total infant body weight within 24-hrs of delivery).

We used the Multivariate Nutrient Density Model approach, as described by Hu, et al[55] to estimate the effects of maternal macronutrient intake on maternal and neonatal outcomes. This method was used, as it is a well-established way of assessing diet composition as the exposure of interest. In this approach, each macronutrient is characterized as its percent of total energy (calories) contributed; one macronutrient is omitted from the model, and total calories consumed (kcal) is included as a covariate. Macronutrient coefficients are interpreted as the estimated effect of isocaloric substitution of the macronutrient of interest for the omitted macronutrient (in this case, protein). In this model total calories is kept in the model as a covariate and is interpreted as the impact of total caloric intake from all macronutrients (including protein) on the outcome of interest when all other covariates are held constant. Interpretation of the exposure variables is done independent of one another and is the association of that macronutrient with the outcome of interest, when all other macronutrient and total calories are held constant.

Studies in both human and animal models have demonstrated an association between poor protein intake in pregnancy and adverse outcomes, including low birth weight, impaired metabolic functioning and poor brain development.[5,56,3] This body of research has led to the development of guidelines regarding recommended intake of protein during pregnancy.[6,9] This same level of understanding and recommendations regarding fat and carbohydrate intake during pregnancy do not exist. To better characterize the association between these two macronutrients and maternal and infant outcomes, fat and carbohydrates were selected as the exposures in our regression models.

We examined three outcomes: GWG (continuous), GWG (categorized according to adherence to IOM recommendations), and neonatal adiposity (percent infant body fat, continuous), (Table 1). Exposure variables for all three models were dietary factors, as previously described, including percent calories from fat, percent calories from carbohydrates and total calories consumed (kcal). *A priori* potential confounders were identified based on literature review suggesting important relationship of confounders with both exposures and outcomes, [27,51,57] but not within the causal pathway.[58] Potential confounders included maternal age, maternal pre-pregnancy BMI (continuous) and parity (dichotomized). Linear regression models followed the form shown in Figure 2.

Table 1. Summary of independent and dependent variables and variable type.

Category	Variable Name	Type of variable
Outcomes	Gestational Weight Gain (kg)	Continuous
	Gestational Weight Gain (Categorized)	Categorical 0:Below recommendation* 1: Within recommendation 2: Above recommendation
	Infant adiposity (percent)	Continuous
Exposures	Percent of calories from fat	Continuous
	Percent of calories from carbohydrates	Continuous
	Total calories (kcal)	Continuous
Candidate confounders	Maternal BMI	Continuous
	Parity	Dichotomous
	Maternal age	Continuous

*Recommendation based on BMI-specific gestational weight gain recommendations.

$$Dependent\ variable = \beta_0 + \beta_1(\%\ cal\ from\ fat) + \beta_2(\%\ cal\ from\ carb) + \beta_3(total\ cal) + \beta_4(*)$$

$\boldsymbol{\beta_4}(*) =$ variables based on model building

Figure 2. Full model with isocaloric interpretation, percent energy from carbohydrates (calories), with total calories included as covariate, other confounders denoted as $\beta_4(*)$.

Given the approximate normal distribution of continuous outcomes (GWG and neonatal fat), (Figure 3) linear regression was considered appropriate. Categorized GWG was modeled using polytomous/multinomial logistic regression, with "within recommendation" as the referent outcome.

Linearity assessment. In crude models, linearity of the relationships between continuous independent variables and each dependent variable were assessed using both graphical and statistical strategies. This was done using locally weighted regressions and a nonlinearity check diagnostic (nlcheck Stata). Quadratic and cubic terms were also tested.

For the GWG and neonatal fat models, all continuous variables were modeled as linear terms, except BMI, which was included as squared term given significance of the higher-order term ($p<0.01$). Cubic terms for percent calories from fat, and percent calories from carbohydrate in GWG model were significant but excluded because visual inspection suggested that nonlinearity was due to two influential data points and model fit was not improved by including higher order terms for calories from fat, carbohydrates or total calories.

The same linearity assessments were performed for the multinomial logistic regression model for categorized GWG. This was done by creating two models: one comparing those categorized as having gained too little weight, against the reference category (those that had gained within the recommended amount), and another model for those women that had gained over the recommended amount compared to the reference category. No evidence of nonlinearity was present, therefore all terms were kept linear.

Outlier detection. Exploration of data showed several candidate outliers. Data for these participants were verified as accurate with the principal investigator. Outliers and influential data points were evaluated by examining residuals, Leverage's and Cook's distance, DFITS and DFBETA and added variable or partial regression plots. Several candidate outliers or influential points were identified with each test, but the same points were not reliably identified by each test. Associations of interest were similar in regressions regardless of whether candidate outliers were included or excluded, therefore we retained all data points in the model.

Variable selection. Each model's covariates were selected *a priori* based on clinical significance, as previously stated. In addition, we conducted an empirical confounding

assessment using a backward elimination strategy with a 10% change in estimate (coefficient) criterion for the exposures of interest, percent calories from fat and carbohydrates. This was done for both continuous outcomes, GWG and NF (Tables 2 and 3). Precision of the estimate was determined using the Confidence Interval Difference (CID) for the linear regression models. CID is calculated by subtracting lower bound of 95% confidence interval (CI) for coefficient of interest from upper bound of CI, decreased precision is associated with a greater CID. Each confounder resulted in a greater than 10% change in estimates, and precision of the associations of interest did not change substantially with the exclusion of any one variable; thus our empirical confounding assessment supported our *a priori* confounders. Given results for GWG as a continuous outcome, *a priori* selection of variables was used for multinominal logistic regression model and no empirical confounding assessment was done.

Table 2. Assessment of confounding for each independent variable for GWG

	Gestational Weight Gain (continuous)		
	Coefficient (95% CI)	CID	Change in estimate (|%|)
Full model			
Calories from fat	-0.02 (-0.69, 0.66)	1.35	N/A
Calories from carbohydrates	0.35 (-0.24, 0.94)	1.18	N/A
Full-(BMI+BMI^2)			
Calories from fat	-0.09 (-0.92, 0.73)	1.70	350
Calories from carbohydrates	0.38 (-0.34, 1.10)	1.44	9
Full-Parity			
Calories from fat	0.02 (-0.65, 0.69)	1.34	200
Calories from carbohydrates	0.37 (-0.22, 0.95)	1.17	6
Full- Maternal age			
Calories from fat	0.03(-0.62, 0.67)	1.29	250
Calories from carbohydrates	0.40 (-0.15, 0.95)	1.10	14

Coefficient (95% CI), confidence interval difference (CID) and absolute change in estimate (%).

Table 3. Assessment of confounding for each independent variable for neonatal fat (percent)

	Neonatal fat, percent (continuous)		
	Coefficient (95% CI)	CID	Change in estimate (|%|)
Full model			
Calories from fat	-0.001 (-0.005, 0.003)	0.008	N/A
Calories from carbohydrates	-0.001 (-0.005, 0.002)	0.007	N/A
Full-(BMI)			
Calories from fat	-0.001 (-0.005, 0.003)	0.008	15
Calories from carbohydrates	-0.001 (-0.004, 0.003)	0.007	8
Full-Parity			
Calories from fat	-0.002 (-0.005, 0.004)	0.009	30
Calories from carbohydrates	-0.001 (-0.005, 0.003)	0.008	3
Full- Maternal age			
Calories from fat	-0.002 (-0.006, 0.002)	0.008	65
Calories from carbohydrates	-0.002 (-0.005, 0.003)	0.008	72

Coefficient (95% CI), confidence interval difference (CID) and change in estimate (%).

Model Diagnostics: Linear regression models (GWG and neonatal fat). Linearity of both GWG and neonatal fat was confirmed with residuals (ordinary, studentized, jackknife) plotted against fitted values and augmented component plus residual plots. Normality of residuals was assessed with Q-Q, P-norm plots and Shapiro-Wilk W test of residuals; we also assessed normality of outliers using the inter-quartile range (IQR) test.[59] Homoscedasticity of residuals was checked using graphical methods, including residual versus fitted plots as well as numerical tests, including White's test and the Breusch-Pagan test. Variance Inflation Factor (VIF) was used to assess for collinearity within the model; variables having VIF greater than 10 were further considered for collinearity. This was confirmed by assessing the condition number for each model. Model specification was tested using Link test, as well as the regression specification error test.

GWG (continuous) model diagnostics show that the model fits the data well and meets the assumptions required for linear regression modeling (linearity, normality, homoscedasticity, independence and model specification). For neonatal fat percent (continuous), however, the model was found to explain a relatively low amount of variance,

17% (R^2 0.17, Root MSE 0.03) with the inclusion of our selected independent variables (F-stat 1.14, p >0.1). Additionally, results of model specification testing suggest that the model either includes erroneous variables or is missing critical variables. This is based on the results of the Link test, which suggests that there may be missing variables from the model. However, the Regression Specification Error Test (RESET) was negative. These contradictory results suggest that there may be an error in the specification of the model, but there is not enough evidence, based on the RESET test to reject the null hypothesis that there are no omitted variables. It is plausible that these results are due to the fact that the exposures selected are not associated with neonatal adiposity, in contrast to our hypothesis. This is supported by the relatively low amount of variability in neonatal adiposity that is explained by the model.

Model diagnostics: Multinomial logistic regression model (adherence to GWG recommendations). Model specification was assessed using Link test, which demonstrated significant errors in model specification; suggesting either an erroneous variable in the model or a crucial variable missing from the model. Goodness of fit was assessed using the Hosmer-Lemeshow (4.8, p>0.7) which suggests that the model is actually a reasonably good fit.. The Log Likelihood ratio (7.4 p>0.1) and Pseudo R^2 (0.08) suggest that the model does not explain much of the variability in adherence to GWG recommendations, with the variables in the model. The results of the goodness of fit tests, as well as the Likelihood Ratio and pseudo-R2 tests, show that the model fits the data well; yet maternal diet does not explain adherence to GWG recommendations as previously thought, consistent with the small amount of the variability explained by the model (Likelihood Ratio). It is also possible that our relatively small numbers in each category of GWG is contributing to the small

amount of variance explained by the model. Collinearity was assessed using VIF, and indicated no concerns for collinearity.

Section 3. Results

Descriptives and Crude Relationships

Enrolled women had a pre-pregnancy BMI that placed them in the normal (n=10, BMI 18.5-24.9 kg/m^2), overweight (n=15, BMI 25-29.9 kg/m^2) or obese (n=15, BMI >30 kg/m^2) category, as calculated from height and weight from medical records. In terms of racial/ ethnic makeup of participants enrolled in the study (data not shown), thirty one women were White/Caucasian (76%) and 35 identified as Non-Hispanic (85%). The racial make-up of the remaining 24% of women included women who identified as African American, Asian American American Indian/Alaskan native and Pacific Islander.

Prior to pregnancy, 26, 37, and 37% of the study participants were normal weight, overweight, and obese, respectively (Table 4). Women were relatively evenly divided across BMI-specific gestational weight gain category, from below (12), to within (13) and above (16). The mean gestational weight gain for all women was 10.5 kg ($\pm$6.7 kg), ranging from a loss of 13.5 kg to gaining 30.7 kg. The majority of women were nulliparous and not of advanced maternal age. The mean caloric intake for all women was 2400 kcal, with an average of 34% percent of calories coming from fat and 51% from carbohydrates

Table 4. Characteristics of study population

	Count	Percent
Pre-pregnancy BMI (kg/m^2)		
Normal (<25)	11	26
Overweight (25-30)	15	37
Obese (>30)	15	37
Parity		
Nulliparous	24	59
Multiparous	17	41
Maternal age (years)		
Non-AMA* (<35)	30	74
AMA (≥35)	11	26
BMI-specific GWG category		
Below	13	32
Within	12	29
Above	16	39
	Mean (±SD)	
Outcomes		
GWG (kg)	10.5 (±6.7)	
Neonatal Fat (%)	0.43(±0.2)	
Dietary Exposures		
Total calories (kcal)	2382 (±556)	
Percent calories from fat	34 (±7)	
Percent calories from carbohydrates	51 (±8)	

*AMA: Advanced Maternal Age, classified as those older than 35 years at the time of delivery.[60]

Distributions of continuous outcomes (GWG and Neonatal Fat) were assessed to ensure that the use of linear regression modeling was appropriate (Figure 3).

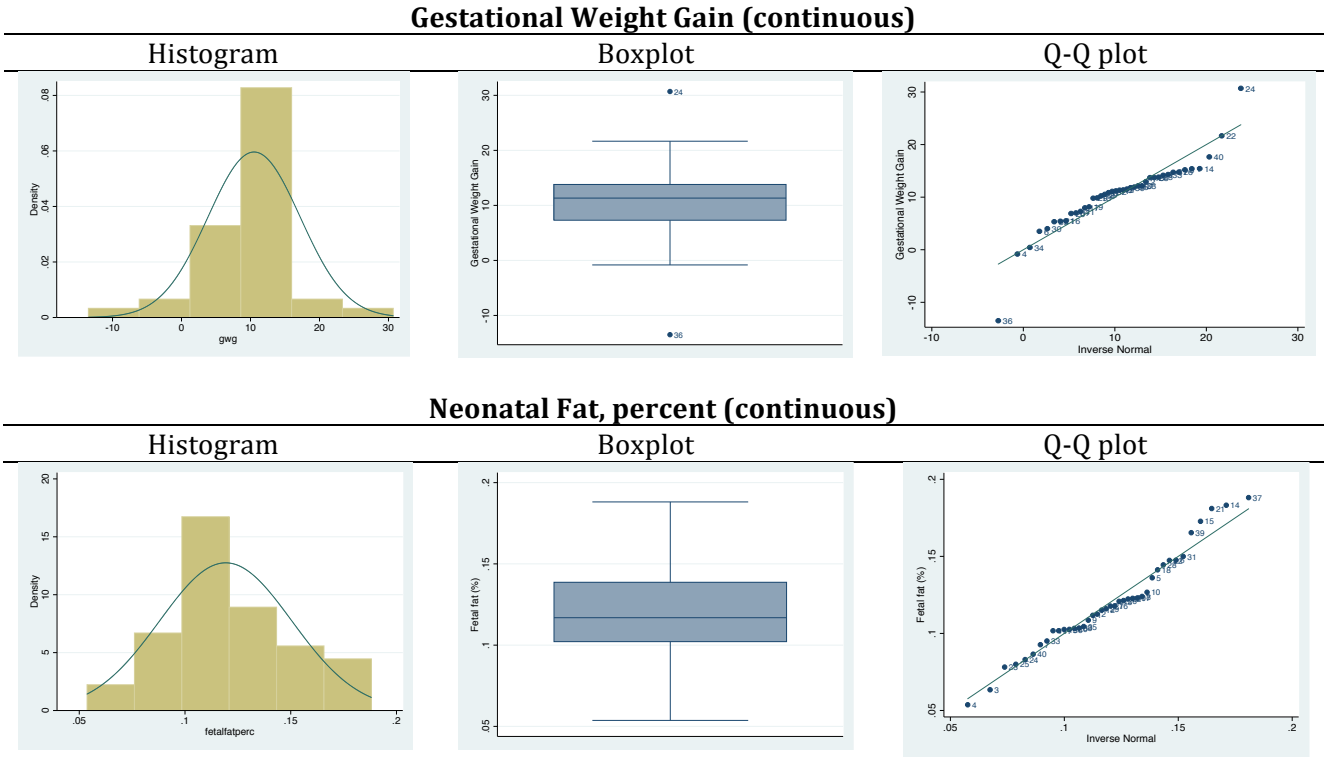

Figure 3. Distribution of Gestational Weight Gain (continuous) and Neonatal Fat represented with histograms, box plots and QQ plots.

GWG (kg) was negatively correlated with percent calories from fat (-0.35) and BMI (-0.48) and positively correlated with percent calories from carbohydrates (0.38) and total calories (0.20); however only BMI, calories from fat and carbohydrates were statistically significant ($p<0.05$) (Table 5). Mean GWG also differed across categories of BMI (ANOVA, $p=0.01$), with women who were overweight gaining, on average, 13.7 kg during pregnancy, compared to 11.3 kg for women with normal BMI, and 6.8 kg for women who were obese, Table 5. This suggests that higher pre-pregnancy BMI is correlated with less GWG. Of note, GWG and the crude relationship to pre-pregnancy BMI was influenced by at least one woman who was obese and lost a significant amount of weight during her pregnancy.

However, based on assessment of potential outliers and influential points, as discussed previously, it was determined that this individual should be kept in the model.

Table 5. Correlations between independent and dependent variables and summary statistics of GWG and neonatal fat across pre-pregnancy BMI category and parity.

	GWG (kg)	**Neonatal Fat (Percent)**
	Correlation with GWG (p-value)	Correlation with neonatal fat (p-value)
Calories from fat	***-0.35 (0.02)***	-0.02 (0.90)
Calories from carbohydrates	***0.38 (0.02)***	-0.03 (0.85)
Total calories	0.20 (0.21)	0.03 (0.86)
Maternal age	-0.11 (0.51)	0.16 (0.32)
Pre-pregnancy BMI (kg/m^2)	***-0.48 (<0.01)***	0.16 (0.31)
Pre-pregnancy BMI	Mean GWG (kg) ±SD	Mean Neonatal Fat (%) ±SD
Normal (n=11)	11.3±2.4*	0.10 ±0.02
Overweight (n=15)	13.6±6.5	0.12 ±0.03
Obese (n=15)	6.8±7.5	0.13 ± 0.04
Parity	Mean GWG (kg) ±SD	Mean Neonatal Fat (%) ±SD
Nulliparous (n=24)	10.6 ±7.8	0.11 ±0.03**
Multiparous (n=17)	10.3 ±5.0	0.13 +0.03

*p=0.01 for ANOVA comparing GWG across category of pre-pregnancy BMI
**p<0.05 for ANOVA comparing neonatal fat percentage across level of parity

Neonatal fat percent (continuous) significantly differed across levels of parity based on ANOVA ($p<0.05$), Table 5, suggesting that as women have more pregnancies there is a correlated increase in neonatal adiposity of their offspring. No difference was seen in neonatal fat percentage across pre-pregnancy BMI categories for mothers, which varied from 12% in infants born to overweight mothers, to 13% in those born to obese women and 10% in those women with a normal pre-pregnancy BMI ($p>0.1$). The lack of correlation between dietary exposures suggests that changes in diet composition are not correlated with changes in infant adiposity prior to controlling for maternal factors including parity, age and pre-pregnancy BMI.

Adherence to GWG recommendations was not associated with parity (Fisher's exact p>0.1). No significant differences were seen for continuous variables across level of GWG category based on ANOVA (p>0.05)(Table 6).

Table 6. Summary of independent variables across GWG category

	GWG		
	<Recommended (n=13)	Within recommended (n=12)	>Recommended (n=16)
Independent Variable	Mean (± SD)	Mean (± SD)	Mea (± SD)
Percent calories from fat	0.35 (± 0.1)	0.35 (±0.1)	0.33 (±0.1)
Percent calories from carbohydrates	0.48 (±0.1)	0.51 (±0.1)	0.52 (±0.1)
Total calories	2297(± 349)	2448 (± 738)	2401 (± 565)
Maternal age	31 (± 5.4)	31 (± 6.4)	31 (± 6.1)
Pre-pregnancy BMI (kg/m^2)	29.8 (± 9.8)	27.5 (± 5.6)	31.3 (± 5.3)

Mean (±SD) for continuous independent variable, across each category of adherence to GWG recommendations.

Estimated effects of maternal diet on GWG

In crude regression analysis, greater percent of calories from fat and higher pre-pregnancy BMI were associated with lower GWG, while greater percent of calories from carbohydrates was associated with higher GWG (Table 7). Estimates from the crude model indicate that a one percent difference in calories consumed from fat is associated with a 0.34kg lower GWG, whereas a one percent difference in percent of calories from carbohydrates is associated with a 0.32kg greater GWG. Total calories, maternal age and parity were not associated with GWG (Table 7). In the adjusted model, maternal diet during the third trimester was not associated with GWG (Table 7). Higher pre-pregnancy BMI was associated with less GWG while maternal age and parity were both unassociated with GWG. The full model explained 56% of the variance in gestational weight gain (model F-stat, p <0.001). Complete model reported in Appendix B, Model 1.

Table 7. Association of gestational weight gain (continuous) with third trimester diet, maternal pre-pregnancy BMI, age and parity [GWG, kg (95% CI)].

	Crude model* GWG, kg (95% CI)	**Adjusted model** GWG, kg (95% CI)
Calories from fat	***-0.34 (-0.64, -0.05)***	-0.02 (-0.69, 0.67)
Calories from carbohydrates	***0.32 (0.07, 0.57)***	0.35 (-0.24, 0.94)
Total calories (kcal)**	1.19 (-0.71, 3.11)	***1.68 (0.02, 3.34)***
BMI	***-0.45 (-0.71, -0.18)***	-0.18 (-0.47, 0.11)
BMI^2	-0.06 (-0.09, -0.03)	***-0.04 (-0.07, -0.01)***
Maternal age	-0.12 (-0.49, 0.25)	-0.08 (-0.38, 0.23)
Parity	-0.30 (-4.65, 4.04)	-1.76 (-5.49, 1.98)

Results of crude and adjusted models for GWG (continuous), Significant results ($p<.05$) are italicized and bold.
*Coefficients for crude models calculated using individual models for each variable with GWG as the outcome.
**Total calories mean-centered to 2300 kcal

Adherence to GWG recommendations was unrelated to all independent variables (Table 8). The likelihood ratio chi^2 of the final model was 7.4 ($p=0.8$). For calories from fat and calories from carbohydrates, odds ratios (ORs) ranged from 0.8 to 0.9 in relation to gaining above vs. within the recommended amount, as well as to gaining in excess vs. within the recommended amount of weight. This suggests that maternal diet during the third trimester is not associated with gaining less or in excess of GWG recommendations.

Table 8. Summary of logistic regression model for gestational weight gain categories

	Gestational Weight Gain (categorical)	
	Under vs. Within Odds Ratio (95% CI)	Over vs. Within Odds Ratio (95% CI)
Variables		
Calories from fat	0.8 (0.5, 1.1)	0.8 (0.6, 1.2)
Calories from carbohydrates	0.8 (0.5, 1.1)	0.9 (0.7, 1.2)
Total calories (kcal)	0.6 (0.2, 1.6)	1.0 (0.4, 2.4)
BMI	1.1 (0.9, 1.2)	1.1 (1.0, 1.3)
Parity	1.3 (0.2, 9.8)	1.3 (0.2, 8.4)
Maternal age	1.0 (0.8, 1.1)	1.0 (0.9, 1.2)

Odds ratios (OR) and 95% CIs for multinomial logistic regression model comparing those that gained above and below the recommended amount of wait, to the referent category, those that gained within recommended amount for their pre-pregnancy BMI.

Estimated effects of maternal diet on neonatal fat

In crude models, diet during the third trimester of pregnancy was not significantly associated with neonatal fat percentage (Table 9). Having a previous pregnancy was associated with greater infant adiposity, with multiparous women having 10% greater neonatal fat than women who had never had a prior pregnancy. Interestingly, BMI was also not associated with greater infant adiposity in these crude association models.

When adjusted for maternal pre-pregnancy BMI, maternal age and parity, diet during the third trimester of pregnancy remained not significantly associated with neonatal adiposity. The fully adjusted model ($p= 0.5$, R^2 0.14) did not significantly predict the outcome of interest (neonatal fat percentage) and accounted for only 14% of the variance in neonatal fat.

Table 9: Association of neonatal fat (percent) with third trimester diet, maternal pre-pregnancy BMI, age and parity, [Neonatal fat % (95% CI)]

	Crude model Neonatal fat % (95% CI)]	**Adjusted model** Neonatal fat % (95% CI)]
% calories from fat	<-0.01 (<-0.01, 0.01)	<-0.01 (<-0.01, 0.01)
% calories from carbohydrates	<-0.01 (<-0.01, 0.01)	<-0.01 (<-0.01, 0.01)
Total calories (kcal)	<0.01 (<-0.01, 0.01)	<0.01 (-0.01, 0.01)
BMI	<0.01 (<-0.01, 0.01)	<0.01 (<-0.01, 0.01)
Maternal age	<0.01 (<-0.01, 0.01)	<0.01 (<-0.01, 0.01)
Parity	***0.1 (<0.01, 0.20)***	0.02 (<-0.01, 0.04)

Results of crude and adjusted linear regression models assessing association between maternal diet and Neonatal fat (%). Significant results (p<.05) are italicized and bold.

Section 4. Discussion

We found that maternal consumption protein, fat and carbohydrates, in the third trimester of pregnancy was not associated with GWG or adherence to the 2009 IOM guidelines for GWG. We also found maternal diet during the third trimester of pregnancy to be unrelated to infant adiposity measured around the time of birth.

Maternal Dietary Predictors of GWG

Our findings suggest that a woman's dietary decisions regarding calories consumed in the form of fats, protein or carbohydrates during pregnancy have less impact on weight gained during this time than is often assumed. Evidence of the association between diet and GWG remain limited, although several studies have shown that greater intake of sweets, fried foods, dairy and total calories was related to increased GWG.[29,61,62] However, studies evaluating the role of proteins and carbohydrates have had conflicting results, suggesting that greater glycemic load and/or index is related to greater or less GWG,[63] or is unrelated to this outcome.[32,64] The literature also suggests that fat is likely associated with GWG;

however, the type of fat may determine the directionality of this relationship and likely contribute to the lack of consistency in the body of evidence.[33]

A study done in 2004, by Lagiou, et al. demonstrated a positive correlation with intake of proteins and animal-derived lipids in the second trimester of pregnancy and GWG, whereas an inverse relationship was seen between carbohydrate intake and weight gain. Of note, this study examined these relationships independent of one another, not taking into account the overall relationships and potential confounding between these nutritional components.[31] Research conducted since has demonstrated contradictory findings, including increased GWG in association with increased carbohydrate[61,62] and an inverse relationship between protein consumption and GWG.[63] A recent meta-analysis conducted in 2016 concluded that there is a dearth of high-quality data about the role of macronutrient intake on the likelihood of gaining excess weight during pregnancy[33]and that among those studies that are of high-quality there remains a considerable amount of discordance in their findings. This, in conjunction with the conflicting evidence cited, demonstrates the need for further research in this area.

An important alternative explanation for our findings is the impact of various types of fats and sugars on GWG. These components of diet, alone, or in conjunction with macronutrients may explain a larger amount of the variation in GWG than considering the broad categories of macronutrients as was done in our study. Evaluating the role of fats and sugars in GWG may elucidate some nuanced relationships not fully appreciated when grouped as macronutrients. While Tryggvadottir, et al. showed an association between saturated fats and the development of gestational diabetes (GDM),[65] we found no studies examining similar associations for GWG. While one study found no association between consumption of various types of fats (including poly and monounsaturated) and GWG,[66] the study scope was limited and other studies have since demonstrated an association between

intake of both mono and polyunsaturated fats and greater likelihood of excess GWG.[67] This discrepancy demonstrates the need for high-quality studies to fully describe this relationship. Understanding the role of various types of sugars, fats and potentially micronutrients in the likelihood of gaining excess weight during pregnancy is an essential next step in this body of research.

Additionally, it is notable that while studies examining the relationships between maternal diet have been performed, a meta-analysis done in 2016 by Tielemans, et al, found that only eleven of fifty six studies on this subject were of high-quality[33]. Limitations of other studies have included small sample sizes, lack of adequate control or comparison group and limited data collected regarding dietary, anthropometric measurements and lack of data about infant outcomes. Our study is unique in that it examines high quality dietary measures and includes not only GWG but also infant skinfold measures, an important outcome measure not often available.

Adherence to IOM GWG guidelines was not associated with maternal diet during the third trimester of pregnancy and a woman's likelihood of gaining less, within or below these recommendations. An important consideration when looking at adherence to GWG guidelines is the loss of small variations seen in the data when GWG is kept as a continuous variable. Despite this limitation, GWG categorized based on IOM recommendations continues to be the measure most often used by studies looking at GWG. Additionally, it is notable that when GWG is categorized there is no consideration or change in GWG recommendations for women with starting BMI's in Class II (35-39.9 kg/m^2) or Class III (BMI $\geq$ 40 kg/m^2) obesity categories.

Maternal Dietary Predictors of Neonatal Adiposity

None of the maternal dietary variables or characteristics were associated with neonatal adiposity. Our findings do not support our hypothesis that greater intake of carbohydrates is associated with greater neonatal adiposity. Our finding is inconsistent with previous research showing that maternal consumption of a diet with a lower glycemic index is associated with lower abdominal adiposity in their infants.[68] Others have shown that maternal consumption of sodium and saturated fats in the second and third trimesters of pregnancy is related to higher/lower infant adiposity,[69] but these results have not been replicated.

Overall, numerous questions remain about the role of diet during pregnancy and infant adiposity. Much research to date has focused on birth weight, rather than infant adiposity. Birth weight offers some information about normal growth and development[70] and has been characterized as a risk-factor for long-term health outcomes including diabetes and obesity,[14] however, the correlation between birth weight and infant adiposity is only moderate;[52,53] the latter is a more specific predictor of long-term health outcomes in infants.[17,18,71] Research has consistently demonstrated an association between greater adiposity at birth and elevated risk of obesity, diabetes and cardiovascular disease in later life,[72,73] making infant adiposity an important outcome, and worthy of further research to determine its origins. However, given the lack of association seen in our study and the lack of consensus evidence in the literature about the impact of maternal diet on infant adiposity, it is worth considering the possibility that diet may play a less important role in infant adiposity and that factors such as maternal pre-pregnancy BMI, and genetic factors may play a more significant role in determining infant adiposity than currently thought.

Clinical and Public Health Implications

Clinical guidelines and recommendations for diet during pregnancy assume that weight gained during this time is affected by diet, generally focusing on recommending healthy eating and exercise[47,62,74] to gain within the recommended amount. In contrast, our findings suggest that third trimester maternal diet is unrelated to GWG and related intergenerational outcome, neonatal adiposity. If there is, in fact, a weak relationship between maternal diet in late pregnancy and these valuable predictors, there is a need to shift clinical and public health strategies away from focusing on maternal diet choices during late pregnancy and towards pre-pregnancy counseling on healthy eating and exercise and postnatal care. However, a shift of this magnitude in clinical practice requires a clear understanding of the relationships at play; therefore, further research in this area to understand nuanced relationships between diet and these important predictors is needed.

Pregnancy provides a unique opportunity for young women to have frequent contact with the healthcare system and providers, and often is a time when women are highly motivated to make behavior changes to benefit their offspring.[75] This increased contact and motivation during pregnancy allows for improved dialogue and education of potentially at-risk women, offering an excellent opportunity to encourage behavior and lifestyle changes. Although the research is mixed in terms of the efficacy of intervention in pregnancy and the impact on GWG, there is a growing body of evidence suggesting that education and goal-setting strategies focused on diet and exercise in pregnancy do benefit women, decreasing the likelihood of their gaining excess weight during pregnancy.[76,77] In particular these interventions have been found to be most effective in women with obesity who have less education and lower socioeconomic position.[37] However, a necessary component of this dialogue is provision of guidelines that offer relevant and accurate information for the pregnant population. An additional consideration is the growing body

of evidence suggesting that pre-conception counseling that encourages women to attain a healthy weight prior to their pregnancy may offer more benefit in terms of not only GWG but long-term health outcome.[24] That is, diet during pregnancy may, as our study suggests, be unrelated to both maternal weight gain during this time and infant adiposity at the time of delivery.

Although evidence suggesting the importance of exposures *in utero* continues to grow, the body of literature available remains inadequate for developing recommendations for diets that are ideal for both mothers and their infants. Our research contributes to knowledge about the relationship between these factors, offering a novel approach to assessing the role of maternal diet on predictors of long-term health.

Strengths and Limitations

Dietary assessment is inherently challenging to study in observational data given its subjective nature. The use of validated and standardized surveys, such as those used in our study, helped to eliminate some recall bias, as did the administration of surveys by trained staff. Additionally, the use of multiple 24-hour food recalls spanning both weekdays and weekends was a significant advantage to the data collected in our study allowing for closer approximation of true dietary intake during this time period. Although there are inherent risks for bias in self-reported diet measures we attempted to control for these by using repeated measures, trained staff and by spanning both weekdays and weekend days.

While there was no intervention in this study, the standard of care dictates that providers make recommendations for GWG, diet and exercise in pregnancy for all patients, based on pre-pregnancy BMI.[74] This differential treatment may have lead to difference in guidance and diet counseling in women with varying pre-pregnancy BMIs and may have influenced both our exposure and out outcomes. This effect, however, was not measured in

the final study and therefore was not controlled for in the analysis. Another important consideration is that this study was done on individuals receiving their prenatal care at OHSU, which may limit the generalizability of the results to other populations. Other potential confounders, such as socioeconomic status, education, partner/paternal involvement with pregnancy, and social support, were not collected. Despite these limitations, we examined multiple measurements of fine-grained exposure and outcome data. Such data are rarely if ever available in larger datasets (e.g., administrative data), and provide a rare opportunity to examine high quality maternal dietary data in relation to both GWG and infant body composition.

An additional limitation to the study is the modest number of individuals enrolled. While the data collected were of high quality, the small number of participants limits the statistical power to potentially detect an association between the exposures and outcomes being explored. If the lack of association seen in our study were to be seen in a larger study, this would necessitate a paradigm shift in terms of our approach to clinical and public health practices surrounding diet in pregnancy. However, with the limited size of this study we are not yet able to state definitively that there is no association.

Future Research Directions

Additional dietary variables of interest include specific dietary sugars (fructose, maltose, dextrose, sucrose, galactose, starch, and glucose), fiber (soluble and insoluble), cholesterol, and mono and polyunsaturated fats. Micronutrients are of interest when considered in concert with the intake of macronutrients and their relationships with pregnancy and birth outcomes. Micronutrients as well as various sugars and fats have been investigated as important contributory factors in weight gain in the non-pregnant

population,[78] [79] [80] as well as in limited research in the pregnant population, however further evaluation of these relationships may offer better insight into strategies for counseling patients about dietary choices. In addition, enrolling women in the pre-conception period and first trimester of pregnancy would give additional insight into the role of early dietary choices on GWG and neonatal adiposity.

Conclusions

GWG and infant adiposity are two important predictors of long-term health effects in mothers and infants.[81] The body of literature has yet to fully describe the role of maternal diet with respect to these two variables, which limits knowledge on one key, potential modifiable driver of these outcomes. In our pilot study, maternal diet during the third trimester of pregnancy was unrelated to GWG. This lack of an association was seen when GWG was kept as a continuous variable or when it was broken down into categories of adherence to GWG recommendations. Additionally, we did not find an association between maternal diet during the third trimester of pregnancy and infant adiposity at the time of birth. These results begin to address some of the important questions regarding the role of maternal diet during this crucial time period.

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Appendix A.

Table A. Inclusion/Exclusion criteria

Inclusion/Exclusion Criteria for MBC **Study**		
	Inclusion	**Exclusion**
Term pregnancy	≥ 37 weeks	< 37 weeks
Age	>18 years	<18 years
Consent	Willing and able to consent	Unable to consent
Gestation	Single	Multiple
Hepatitis or HIV infection	No history of either	History of one or both
Congenital anomalies	None documented	Documented
Social history	No drug or alcohol abuse history	History of drug or alcohol abuse
Inflammatory illness	No history of	History of
Chronic illness	No history of	History of
Rheumatologic disease	No history of	History of
Diabetes	No history of	History of

Inclusion and exclusion criteria for women meeting minimum requirements for involvement in original MBC study. Each value was checked using EMR and was validated with each participant during their pre-enrollment visit to OHSU.

Table B. Medical/ pregnancy history questionnaire.

Medical/ Pregnancy History
Estimated date of conception
Pre-pregnancy weight (kg)
Height
BMI (Body mass index, kg/m^2)
1st prenatal visit weight (kg)
12-14 week weight (kg)
26-28 week weight (kg)
Parity
Fasting glucose
1 hr Glucose Tolerance Test (GTT)
3 hr GTT
Estimated neonatal weight (grams)
Abdominal circumference (fetus)
Head circumference (fetus)

Data collected from all women meeting inclusion criteria for original MBC study. Confirmed using data from EMR.