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Associations of Maternal Diet and Placenta Leptin Methylation

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Abstract

Background: Maternal diet is an important factor in prenatal development that also has implications for disease risk later in life. The adipokine leptin is a key regulator of energy homeostasis and may be involved in the association between maternal nutrition, maternal obesity, and infant outcomes. DNA methylation of placenta genes may occur in response to exposures and may program subsequent infant development. This study examined maternal diet, placenta leptin gene DNA methylation, and neonatal growth in a sample of healthy neonates and their mothers.

Methods: Mothers and their healthy neonates (N=135) were recruited within 1–2 days following delivery at Women and Infants Hospital in Providence, RI. A structured interview was conducted to assess maternal dietary intake. Maternal pre-pregnancy weight, weight gain during pregnancy, maternal health, medications, and vitamin use were obtained from medical records. Bisulfite pyrosequencing was used to measure methylation of CpG sites in the promoter region of the placenta leptin gene and determine genotype of the leptin single nucleotide polymorphism (SNP) rs2167270, which is known to influence leptin methylation. Bivariate analyses and linear regression models were used to evaluate associations of demographics, diet, and mean leptin methylation.

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CONFLICTS OF INTEREST

All authors have nothing to disclose. All authors report no conflict of interest.

Results: Genotype was a significant predictor of placenta leptin DNA methylation (p<.01), and after controlling for this and other relevant maternal and infant covariates, lower levels of leptin methylation were significantly associated with greater intake of carbohydrates (p<.05), in particular added sugars (p<.05) and white/refined carbohydrates (p<.05). Total caloric intake was also associated with placenta leptin methylation (p<.05), however after controlling for relevant covariates, significance diminished to trend-level. There were no significant associations of placenta leptin methylation and intake of protein (p>.05) or fat (p>.05).

Conclusion: These findings underline the importance of intake of carbohydrate consumption for methylation of the placenta leptin gene. Because methylation reduces gene transcription, lower methylation may indicate a placenta response to high caloric intake and carbohydrate food that would result in higher levels of this hormone during fetal development. Further investigation of the developmental ramifications of epigenetic changes to placenta leptin methylation should be pursued.

Keywords

Leptin; Placenta; Maternal Nutrition; Diet; Epigenetics; Methylation

INTRODUCTION

Early environment plays a critical role in modulating risk for downstream chronic disease. Developing tissues and organ systems *in utero* are particularly susceptible to environmental perturbations that can have long-term consequences for health and disease. Adverse prenatal circumstances, such as reduced nutrient availability, are major risk factors for the future development of metabolic, cardiovascular, neuropsychiatric, immunologic, and gastrointestinal disorders (Ravelli, van Der Meulen, Osmond, Barker, & Bleker, 1999; Roseboom et al., 2001). Further, the fetal overnutrition hypothesis suggests that maternal fuels are in greater abundance in maternal obesity and gestational diabetes mellitus (GDM), predicting excessive nutrient availability after birth, with high fat and high caloric diets yielding increased adiposity and inflammation in offspring (Nicholas et al., 2016; Sullivan, Riper, Lockard, & Valleau, 2015). Recent epidemiological evidence has shown that maternal hyperglycemia is associated with increased risk of abnormal glucose tolerance, obesity and elevations in blood pressure in children by the age seven—effects that were independent of maternal obesity, birth weight, and body mass index (BMI) (Tam et al., 2017).

Prenatal environments are hypothesized to influence downstream health outcomes through a number of mechanisms. Epigenetics refers to a collection of processes that allow for transient environmental influences to have long-lasting effects on DNA expression that endure across subsequent cell generations. Through various means of controlling gene transcription, epigenetic regulation allows for a wide range of phenotypes to arise from identical genomic information. The most stable epigenetic mechanism is methylation of the 5’ cytosine nucleotide within CpG islands, which serves to repress mRNA expression (Smith & Ryckman, 2015).

Leptin is a pro-inflammatory cytokine primarily produced by adipose tissue (also referred to as an adipokine), but also in other tissues, including the placenta (Ashworth et al., 2000).

Leptin plays a central role in the regulation of food intake behaviors, metabolism, and inflammation (Friedman, 2011; Harris, 2014). Centrally, leptin acts as a satiety signal through suppression of orexigenic peptides at the ventromedial hypothalamus (Ahima, 2006). Peripherally, leptin serves to mobilize stored energy through lipolysis (Ahima, 2006). Leptin levels vary with energy states and adiposity—with high levels of leptin in obesity leading to central leptin insensitivity at the level of the hypothalamus (Engin, 2017). Leptin may have particular importance in the perinatal period. Leptin is produced by the placenta and circulating maternal leptin increases throughout the second trimester, producing a physiologic hyperleptinemic state with associated hypothalamic leptin insensitivity to allow for increased appetitive behaviors (Ashworth et al., 2000). Leptin injection at birth in leptin-deficient mice influenced hypothalamic connectivity, resulting in reduced food intake and attenuated weight gain over the lifetime (Bouret, Draper, & Simerly, 2004).

Dysregulated maternal metabolic states, such as pre-pregnancy obesity and GDM, are associated with changes in placenta leptin methylation. Obesity in pregnancy is associated with higher maternal circulating leptin and placenta leptin production has been shown to increase with BMI in non-obese mothers as well. Pre-pregnancy obesity is associated with broad changes in placental gene expression and in particular, reduced placenta leptin expression (Tessier, Ferraro, & Gruslin, 2013). Pre-pregnancy obesity and GDM have both been found to have associations with changes in leptin methylation patterns (Lesseur, Armstrong, Paquette, et al., 2014; Lesseur & Chen, 2018; Nogues et al., 2019).

Further, maternal fasting glucose has been directly correlated with cord blood leptin (Allard et al., 2015). As the blood supply within the placenta can be divided into maternal and fetal systems of circulation, measures of leptin methylation from each side can be compared. For example, maternal glucose levels are positively correlated with maternal side placenta DNA methylation and negatively correlated with methylation in the fetal side (Bouchard et al., 2010).

In order to better understand the contribution of maternal diet to fetal metabolic programming through epigenetic changes in utero, we studied maternal diet and placenta leptin methylation in a group of mother-infant dyads in the immediate postpartum period.

METHODS

Subjects

Subjects (N=135) were mother-infant pairs recruited following delivery at Women and Infants Hospital in Providence Rhode Island, USA as part of the Rhode Island Child Health Study (Marsit, Maccani, Padbury, & Lester, 2012). The current sample, however, is distinct from that used in a previous publication on leptin methylation that did not assess dietary intake, the main focus of the current study (Lesseur, Armstrong, Paquette, et al., 2014). Briefly, term infants born small for gestational age (SGA; $<10^{th}$ percentile) and large for gestational age (LGA: $>90^{th}$ percentile), according to the Fenton growth charts (Fenton, 2003) were gender- and gestational age-matched with infants that were appropriate for gestational age (AGA). Post-recruitment, all infants were re-classified into birth weight categories using recently developed growth charts (Fenton & Kim, 2013). Exclusion criteria

included non-singleton pregnancy, maternal age <18 years, maternal life-threatening complications and infant congenital or chromosomal abnormalities. All mothers provided written informed consent following protocols approved by the Institutional Review Board at Women and Infants Hospital and Dartmouth College.

Procedures

Dietary Intake—A brief structured interview was developed in our laboratory to measure maternal dietary intake in the third trimester, and all interviews were administered by a single trained research assistant. The interview asked about the consumption of 60 categories of common food and beverage items in an average week over the past three months (third trimester). These food items included: milk (fat-free, low fat, or whole), yogurt (fat-free, low fat, or whole), cheese (fat free, low fat, or whole), ice cream (fat-free, low fat, or whole), bread (whole wheat or white/refined), cereal (whole wheat or white/refined), crackers (whole wheat or white/refined), rice (whole grain or white/refined), pasta, muffins, potatoes, French fries, potato chips, pretzels, red meat, pork, chicken/turkey, fish, eggs, soy/tempeh, beans, nuts, vegetables, fruit, olive oil, corn/vegetable oil, butter, margarine, cookies, candy, cake/pie, soda, diet soda, juice, iced tea and coffee. Frequency of consumption of a standard portion of each food or beverage item consumed was estimated by participants. The reported dietary intake was converted into nutrient intake using macronutrient and calorie values obtained from the U.S. Department of Agriculture, Agricultural Research Service, (USDA) National Nutrient Database for Standard Reference 28 (U.S. Department of Agriculture, 2019). In lieu of detailed information regarding the reported items consumed, representative standards for each food item were selected. Macronutrients assessed were: carbohydrates, sugars, proteins, and fats (lipids). A whole wheat carbohydrate variable was created using the sum of calculating whole wheat carbohydrate intake (included: bread, cereal, crackers, rice). A white/refined carbohydrate variable was calculated using the sum of white/refined carbohydrate intake (included: bread, cereal, crackers, rice, pasta, pretzels, muffins, cake/pie, cookies).

Clinical Information—Clinical information was collected using a structured chart review form and an interviewer-administered questionnaire to gather information on socio-demographic variables, personal and family history of medical conditions, lifestyle and exposures. Self-report of height and weight obtained during interview served to calculate maternal pre-pregnancy body mass index (BMI) as well as maternal gestational weight gain (GWG). Tobacco use during pregnancy, hypertension in pregnancy, type I diabetes mellitus (T1DM), type II diabetes mellitus (T2DM), and gestational diabetes (GDM) were obtained from medical charts.

Placenta Leptin Methylation

Sample collection and nucleic acid extraction—Fetal placental samples were collected within two hours following delivery; twelve fragments, three from each quadrant were obtained two centimeters from the umbilical cord and free of maternal decidua. Collected tissue was placed on RNAlater solution (Life Technologies, Grand Island, NY, USA) and stored at 4°C. Tissue segments were blotted dry, snap frozen in liquid nitrogen, homogenized by pulverization using a stainless-steel cup and piston unit (Cellcrusher, Cork,

Ireland) and stored at −80°C until needed. DNA was extracted from homogenized placental samples using the DNAeasy Blood & Tissue Kit (Qiagen, Inc, Valencia, CA, USA). The resulting DNA was quantified using the ND 2000 spectrophotometer (Thermo Fisher Scientific Inc., Watham, MA, USA). All procedures were performed following manufacturer's protocols.

DNA bisulfite modification and pyrosequencing analysis—DNA samples (500 ng) were sodium bisulfite-modified using the EZ DNA methylation Kit (Zymo Research, Irvine, CA, USA), following the manufacturer's protocols. For methylation detection, bisulfite pyrosequencing was employed. Primers (Integrated DNA Technologies, Inc, Coralville, IA) were designed using the PyroMark Assay Design software version 2.0.1.15 (Qiagen) in a region previously associated with leptin expression (Bouchard et al., 2010; Melzner et al., 2002; Yokomori, Tawata, & Onaya, 2002). The PyroMark PCR kit (Qiagen) and PCR primers (Table S1) were used to amplify a 383 base pair region in the leptin promoter; cycling conditions were 94 °C for 15 min followed by 50 cycles of 94 °C for 1 min, 56 °C for 1 min and 72 °C for 1 min with a final extension of 10 min at 72 °C. Pyrosequencing was performed in triplicate using the Pyromark MD (Qiagen) instrument with two forward assays covering a total of 23 CpG loci (Tables S1 and S2). Non-CpG cytosines within each read served as internal controls to verify bisulfite DNA modification efficiency (>95% in all samples) and each pyrosequencing run included a no template control; all samples were sequenced by the same operator. DNA methylation results were analyzed with the PyroMark CpG software, version 1.0.11 (Qiagen).

SNP genotyping—Samples were genotyped for rs2167270 (+19G > A), a common SNP in the leptin promoter region studied. Genotypes calls were made by analyzing the pyrograms and comparing peak heights for each allele; triplicate wells were called independently and compared for quality control.

Statistical Analysis—All analyses were performed using SPSS, Version 22 (IBM Corporation, Armonk, NY, USA). Pairwise Pearson correlations were used to compare leptin DNA methylation between the 23 CpG loci analyzed. There was a high degree of intercorrelation of the DNA methylation at each of the 23 CpGs (mean r = 0.7); thus we used the mean across the region. Dietary measures were winsorized by transforming values < 5% and > 95% of the score distribution to the closest value within this percentile range. The leptin promoter mean methylation distributions were assessed with Shapiro–Wilk test, confirming normality. Bivariate analyses were performed using a Student's t test, Chi-square 1-way analysis of variance or Pearson's correlation, as appropriate. The Benjamini-Hochberg false discovery rate method was used to correct for multiple comparisons. Associations of methylation and outcomes controlling for relevant covariates were examined using linear regression.

RESULTS

Sample Characteristics

Characteristics of the study population are listed in Table 1. In accordance with the study design, all infants were born at term. Consistent with local demographics, the majority of infants (N=135) were born to white mothers (72.5%) who ranged between 18 and 40 years of age (*M*=30 years).

Maternal Diet

Table 2 displays summary values for maternal macronutrient intake. Women reported consuming an average of 283.0 g of carbohydrates daily (SD = 103.4 g; range = 125.7–660.1 g). Of these, an average of 67.2 g were white/refined (SD = 29.6 g; range = 0.0–232.0) and 27.1 g were whole wheat (SD = 33.3; range = 0.0–169.1). They reported consuming a diet with an average of 134.7 added sugars (SD = 65.6; range = 45.7–398.6), 75.8 g of protein (SD=26.3; range = 25.3–150.5), and 57.8 g of fat (SD = 21.4; range = 11.0–128.1). Mothers reported an average of 1913.8 kilocalories per week (SD = 606.3; range = 721.7–3796.0), a value approximating the recommendation for women in the third trimester of pregnancy of 2,200 kilocalories daily (McGuire, 2011). Bivariate associations among maternal diet variables are presented as Pearson correlations (*r*) in Supplementary Table 1. Dietary measures were highly intercorrelated, with significant associations among diet variables ($p<0.5$), with the exception of carbohydrates with fat or with protein (p's>0.5).

Placenta Leptin DNA methylation

Mean leptin methylation was normally distributed and ranged from 9% to 35%. Genotype frequencies at rs2167270 were in Hardy-Weinberg equilibrium, with 13% of the infants homozygous for the variant allele (A), 48% heterozygous, and 39% homozygous for the dominant allele (G).

Infant and Maternal Predictors of Placenta Leptin DNA methylation

The results of the bivariate analyses between placenta leptin methylation and maternal and infant characteristics are presented in Table 3. As previously reported (Lesseur et al., 2013; Lesseur, Armstrong, Paquette, et al., 2014), placenta leptin gene promoter methylation was higher in infants with the A/A genotype (p=.003). In contrast to the previous study, this distinct sample did not demonstrate a significant difference in leptin gene promoter methylation by infant sex (p=.09), gestational diabetes (p=.74) or maternal pre-pregnancy obesity (p=.64).

Associations of Maternal Diet with Mean Placenta Leptin DNA Methylation, Infant, Maternal Predictors

We examined the associations of maternal dietary variables and mean methylation of placenta leptin DNA (Table 4). Mean placenta leptin methylation was negatively associated with reported caloric intake (r = −.176, p = .041), carbohydrate intake (r = −.221, p = .010), and added sugar intake (r = −.205, p = .017). Further, white/refined carbohydrate (r = −.213, p = .013), but not whole wheat carbohydrate intake (r = .013, p = .881) demonstrated

significant inverse associations with placenta leptin DNA methylation. After correction for multiple comparisons, all of these associations remained significant with the exception of caloric intake. There were no significant associations of placenta leptin methylation with fat ($r = -.055$, $p = .530$) or protein ($r = -.096$, $p = .272$) intake.

Among infant and maternal predictors (Supplementary Table 2), maternal report of fat intake was negatively associated with tobacco use ($t(133)=-2.38$, $p< .05$). Otherwise, there were no significant findings from bivariate analyses of macronutrients (carbohydrate, fat, protein), sugar, and caloric intake with infant and maternal predictors.

Covariate Selection

We evaluated whether a number of infant and maternal variables were associated with placenta leptin methylation, and thus should be included in regression analyses. We examined whether placenta leptin methylation were significantly correlated with rs2167270 genotype, infant sex, birthweight, maternal age, maternal pre-pregnancy obesity, maternal gestational diabetes, gestational weight gain, maternal hypertension, and tobacco use in pregnancy. Maternal age and genotype were both significantly associated with placenta leptin methylation ($r = .17$, $p <.05$ and $t(133)=2.99$, $p<.01$, respectively) and were thus included in the regression model. While maternal pre-pregnancy obesity and gestational diabetes were not significantly associated with placenta leptin methylation in this sample ($t(133)=-.46$, $p >.05$ and $t(133)=-.33$, $p>.05$, respectively), previous findings from a larger sample (Lesseur, Armstrong, Paquette, et al., 2014), did demonstrate an association and were thus included in the model as well.

Main Effects Model

A series of multiple linear regressions (Table 5) was calculated to predict placenta leptin methylation based on dietary variables (carbohydrate, added sugar, white/refined grains, and caloric intake), rs2167270 genotype, infant sex, maternal age, maternal pre-pregnancy obesity, and gestational diabetes. A significant regression equation was found, $F(6,126) = 3.53$, $p = .003$, with an adjusted R^2 of .103, with greater amounts of carbohydrate intake predicting less placenta leptin methylation ($\beta = -.20$, $p = .018$). These effects were observed beyond the influence of genotype ($\beta = .251$, $p = .003$), maternal age ($\beta = .130$, $p = .127$), infant sex ($\beta = -.091$, $p = .287$), gestational diabetes ($\beta = .022$, $p = .796$) and obesity ($\beta = .021$, $p = .802$). Similarly, models including added sugar ($F(6,126) = 3.299$, $p = .005$, adjusted $R^2 = .095$, ($\beta = -.179$, $p = .035$)) and white/refined grains ($F(6,126) = 3.720$, $p = .002$, adjusted $R^2 = .110$, ($\beta = -.223$, $p = .010$)) were predictive of less placenta methylation. However, caloric intake did not significantly influence the model ($F(6,126) = 3.116$, $p = .007$, adjusted $R^2 = .088$, ($\beta = -.179$, $p = .062$)).

DISCUSSION

This study demonstrates that maternal intake of carbohydrates, in particular added sugar and white/refined carbohydrates, are negatively predictive of placenta leptin methylation. These findings withstood correction for other potential mediators of placenta leptin methylation, including genotype, maternal age, infant sex, gestational diabetes, and pre-pregnancy

obesity. While caloric intake was similarly associated with placenta leptin methylation, this correlation did not withstand correction for multiple comparisons. Despite the role of lipids as an energy source, reported fat intake was not associated with leptin methylation. Whole wheat carbohydrate and protein intake were also not associated with placenta leptin methylation. As methylation inhibits gene expression, increased leptin levels would be expected in the developing fetus of mothers with higher carbohydrate intake. Taken together with previous evidence from this study (Lesseur et al., 2013; Lesseur, Armstrong, Paquette, et al., 2014) and others (Dabelea et al., 2000; Group et al., 2008; Wright et al., 2009), metabolic exposures *in utero* appear to influence the fetal epigenetic signature and may have implications for growth (Boeke et al., 2013; Hinkle et al., 2019; Mantzoros et al., 2009) and later-life health outcomes (Li et al., 2019).

The developmental origins of health and disease (DOHaD) paradigm theorizes that resource availability in prenatal environments contributes to cardiovascular and metabolic health outcomes through mechanisms that program the organism in anticipation of a resource rich or poor future (Barker, 2007; Barouki, Gluckman, Grandjean, Hanson, & Heindel, 2012). A foundation of evidence suggests that environments characterized by nutrient availability imbalances and dysregulation of circulating leptin might result in a compensatory response that could influence infant growth and development. Several studies in both animals and humans demonstrate strong associations between maternal nutrition and alterations in postnatal adiposity, appetite, metabolism, and brain function. Nutrient availability in pregnancy is central to fetal growth, with deficiencies in critical periods related to low birthweight, poor skeletal development and changes in adiposity that are associated with later-life metabolic risk (Kennedy et al., 2018; Lillycrop & Burdge, 2012). Similarly, rodent models of pregnant dams fed an obesogenic diet produced offspring characterized by hyperphagia, increased adiposity, decreased muscle mass, reduced locomotive activity and accelerated puberty (Guo & Jen, 1995).

However, the findings in this study do not indicate whether dietary modulation of leptin methylation and expression has significant effects on fetal growth and development. There were no significant associations of placenta leptin methylation and measures of growth in the neonate, including birthweight, length and head circumference (not presented). This may be related to power, as previous studies have found correlations of cord blood leptin with infant growth (Mantzoros et al., 2009). Further investigation of associations of placenta leptin methylation and developmental trajectories is warranted.

While it cannot be assumed that mothers with higher carbohydrate intake had higher circulating blood glucose, it is well-established that maternal hyperglycemia increases risk of both short- and long-term adverse infant outcomes, including premature delivery, shoulder dystocia, neonatal intensive care, hyperbilirubinemia and preeclampsia (Group et al., 2008). Further, maternal GDM has also been associated with increased systolic blood pressure at 3 years-old, an association that appears to be mediated by adiposity (Wright et al., 2009). Metabolic outcomes such as risk for development of T2DM are independent of inherited predisposition (Dabelea et al., 2000), pointing toward *in utero* environmental regulation of metabolic risk.

This question has been pursued by Mantzoros and colleagues, who have followed a large cohort of neonates through adolescence. In their prospective study, they found cord blood leptin levels were directly correlated with birthweight, but negatively associated with growth in both weight and length from 0–6 months. At 3 years of age, these same cord blood levels at birth were negatively associated with BMI (Mantzoros et al., 2009) and in adolescence, they were associated with measures of cardiometabolic risk including increased systolic blood pressure (Li et al., 2019). Further, in a cross-sectional study of 431 adolescents, leptin promoter methylation in salivary DNA was negatively associated with measures of obesity (Dunstan et al., 2017)

We have previously described associations between placenta leptin DNA methylation and neurobehavioral changes characterized by lethargy and hypotonicity, similar to behavioral phenotypes of the leptin deficient ob/ob mice (Lesseur, Armstrong, Murphy, et al., 2014). Increased methylation of the leptin gene in the human placenta is associated with increased lethargy and hypotonicity in male, but not female newborns (Lesseur, Armstrong, Murphy, et al., 2014), supporting not only a connection between epigenetic regulation of the leptin receptor and the relay of important information to the fetal brain, but also a sex-specificity in these outcomes.

This study is limited in its ability to clarify the mechanisms underlying the observed changes in leptin methylation. A measure of maternal serum leptin levels would serve to contextualize the current findings. Additionally, longitudinal follow-up of the infants would provide for improved understanding of growth and neurobehavioral outcomes. The food frequency questionnaire developed for this study is limited in that it relies upon retrospective recall of typical third trimester dietary habits, and uses relatively broad categories of foods. Further, there was no assessment of energy under-reporting or over-reporting and the range of reported weekly caloric intake was broad. This is offset by the finding that associations remained consistent across a number of different types of carbohydrate groups, a larger sample may supplement and fortify the results already obtained and provide further correlations. This study provides promising preliminary data, drawing attention to the relationship between maternal diet and placenta leptin methylation, and its potential role in infant growth and development.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights:

- Maternal intake of carbohydrates is negatively predictive of placenta leptin methylation.
- Among carbohydrates, added sugars and white/refined carbohydrates, but not whole wheat carbohydrates were negatively predictive.
- These findings withstood correction for potential mediators of placenta leptin methylation, including genotype, maternal age, infant sex, gestational diabetes, and pre-pregnancy obesity.
- Maternal intake of fat and protein did not demonstrate associations with placenta leptin methylation.

Table 1:

Study population descriptive statistics

Maternal characteristics		**Infant characteristics**	
Age (years), *M (SD)*	30.5 (5.0)	Gestational Age (weeks), *M (SD)*	39.1 (0.9)
BMI (kg/m^3), *M (SD)*	26.1 (6.9)	Birthweight (kg), *M (SD)*	3.4 (0.6)
Pre-pregnancy obesity, *N (%)*	26 (19.3)	SGA, *N (%)*	18 (13.3)
Gestational Diabetes Mellitus, *N (%)*	12 (8.9)	AGA, *N (%)*	95 (70.4)
Gestational weight gain, *N (%)*		LGA, *N (%)*	22 (16.3)
Inadequate	20 (14.8)	Sex, *N (%) female*	65 (48.1)
Adequate	46 (34.1)	Delivery Method, *N (%) vaginal*	66 (48.9)
Excessive	68 (50.4)	Genotype (rs2167270), *N (%) A/A*	18 (13.3)
Race/Ethnicity	98 (72.6)		
N (%) white	98 (72.6)		
N (%) black	12 (8.9)		
N (%) Hispanic	2 (1.5)		
N (%) Asian/Pacific Islander	4 (3.0)		
N (%) other	17 (12.6)		
Hypertension in pregnancy, *N (%)*	8 (5.9)		
History of type I Diabetes, *N (%)*	0 (0.0)		
History of type II Diabetes, *N (%)*	2 (1.5)		
Tobacco use in pregnancy, *N (%)*	6 (4.4)		

*BMI, body mass index; SGA, small for gestational age; AGA, Appropriate for gestational age; LGA, large for gestational age.

Table 2:

Summary of maternal macronutrient intake

Macronutrient	Mean Daily Intake (g)	Standard deviation (g)	Minimum (g)	Maximum (g)
Carbohydrate	283.0	103.4	125.7	660.1
White/Refined	67.2	39.6	0.0	232.0
Whole Wheat	27.1	33.3	0.0	169.1
Sugar	134.7	65.6	45.7	398.6
Protein	75.8	26.3	25.3	150.5
Fat (lipid)	57.8	21.4	11.9	128.1
Calories	1913.8	606.3	721.7	3796.0

Table 3:

Bivariate analysis of infant and maternal variables and placenta leptin methylation

Maternal characteristics	**Leptin Methylation *Mean (SD)***	**p-value**
Pre-pregnancy obesity		.64
Yes	24.1 (5.6)	
No	23.5 (5.8)	
Gestational Diabetes Mellitus		.74
Yes	24.1 (6.1)	
No	23.6 (5.8)	
Gestational weight gain		.17
Inadequate	21.9 (6.1)	
Adequate	24.7 (5.2)	
Excessive	23.4 (5.9)	
Hypertension in pregnancy		.18
Yes	20.99 (4.1)	
No	23.8 (5.8)	
Tobacco use in pregnancy		.58
Yes	24.9 (3.5)	
No	23.6 (5.9)	
	Pearson Correlation	
Maternal age	.17	.05
Infant characteristics		
Sex		.09
Male	24.5 (6.1)	
Female	22.8 (5.2)	
Birthweight group		.72
SGA	23.7 (5.7)	
AGA	23.5 (5.8)	
LGA	24.5 (5.9)	
Genotype		**.003**
A/A	27.3 (5.9)	
G/A	23.4 (5.2)	
G/G	22.1 (5.9)	

*Placenta leptin methylation reported as mean percent methylation across 23 CpG sites. SGA, small for gestational age; AGA, Appropriate for gestational age; LGA, large for gestational age.

Table 4:

Bivariate associations among maternal diet variables and placenta leptin methylation

Macronutrient	Pearson Correlation	p-value
Carbohydrate	−.22	.01 *
White/Refined Grains	−.21	.01 *
Whole Wheat Grains	.01	.88
Sugar	−.21	.02 *
Protein	−.07	.44
Fat (lipid)	−.07	.40
Calories	−.18	.04

* Association remained significant after correction for multiple comparisons.

Table 5:

Linear Regression results for placenta leptin methylation

	Model 1		Model 2		Model 3		Model 4	
Variable	**β**	**SE**	**β**	**SE**	**β**	**SE**	**β**	**SE**
Genotype	**.25** **	1.40	**.25** **	1.40	**.23** **	1.396	**.25** **	1.41
Maternal Age	.13	.10	.126	.098	.177	.098	.139	.10
Infant Sex	−.09	.975	−.097	.978	−.113	.967	−.091	.99
GDM	.02	1.70	.026	1.704	.018	1.693	.035	1.71
Obesity	.02	1.22	.017	1.227	−.020	1.237	.030	1.23
Carbohydrates	**−.20** *	.001	—	—	—	—	—	—
Sugars	—	—	**−.18** *	.001	—	—	—	—
White/Refined	—	—	—	—	**−.22** *	.002	—	—
Calories	—	—	—	—	—	—	−.16	.0001
Adjusted R^2	.103		.095		.110		.088	
Model F	**F(6,126) = 3.53** **		**F(6,126) = 3.30** **		**F(6,126) = 3.72** **		**F(6,126) = 3.12** **	

Note: GDM = Gestational Diabetes Mellitus

* p<.05

** p<.01