UNIVERSITY OF CALIFORNIA SAN DIEGO

The Effects of Pregravid Body Mass Index and Gestational Weight Gain on Indicators of Placental Health

A dissertation submitted in partial satisfaction of the
requirements for the degree Doctor of Philosophy

in

Public Health (Epidemiology)

by

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2021

The Dissertation of Toni M. Rush is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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Dedication

In recognition of your unwavering support and inspiration; for your dedication to believing in my abilities even when I doubted them; for bringing joy and adventure to my life; for cheering me on at every step of the way from middle school, high school, undergrad, grad school , and more grad school; for encouraging me to follow my dreams…all of them, this dissertation is dedicated to my best friend and husband, Jay Rush.

In recognition of their roles in making me a stronger, braver woman willing to continue moving forward and accomplish her goals; for every laugh and every hug along the way, this dissertation is dedicated to my four, wonderful children: Ganon, William, Xavier, and Mia.

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List of Abbreviations

BMI	Body mass index
CDC	Center for Disease Control
CV	Chronic villitis
DAG	Directed acylic graph
GDM	Gestational diabetes mellitus
GWG	Gestational weight gain
IOM	Institute of Medicine
AGA	Adequate for gestational age
LGA	Large for gestational age
SGA	Small for gestational age
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
UC	Umbilical cord

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Acknowledgments

I would like to acknowledge my family and my friends who supported my decision and encouraged me to throughout this journey

I would like to acknowledge my colleagues and friends in my JDP cohort. Thank you for all the energetic and stimulating discussions in the classroom and over many meals. Having you all in my corner was more supportive than you will ever know.

I would also like to acknowledge all my professors and mentors at SDSU and UCSD. Each one of you provided valuable guidance along the way and have helped shape me into the researcher I am today.

In addition, I would also like to acknowledge Dr. Louise Laurent for welcoming me into her group. Your enthusiasm for research and for the quest of knowledge is infectious which kept me engaged and excited even when times seemed difficult.

Lastly, I would like to acknowledge Dr. Christina Chambers. Thank you for opening the doors to maternal health and perinatal epidemiology. Your willingness to listen and to encourage my ideas as well as your determination to help me find ways to succeed were invaluable.

Chapter 2, in full, is currently being prepared for submission for publication of the material. Rush, Toni M; Laurent, Louise C; Quintana, Penelope J; Jain, Sonia; Thompson, Caroline A; Parast, Mana; Chambers, Christina D. Toni Rush was the primary investigator and author of this material.

Chapter 3, in full, is currently being prepared for submission for publication of the material. Rush, Toni M; Laurent, Louise C; Quintana, Penelope J; Jain, Sonia; Thompson, Caroline A; Parast, Mana; Chambers, Christina D. Toni Rush was the primary investigator and author of this material.

Chapter 4, in full, is currently being prepared for submission for publication of the material. Rush, Toni M; Laurent, Louise C; Quintana, Penelope J; Jain, Sonia; Thompson, Caroline A; Parast, Mana; Chambers, Christina D. Toni Rush was the primary investigator and author of this material.

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Abstract of the Dissertation

The Effects of Pregravid Body Mass Index and Gestational Weight Gain on Indicators of Placental Health

by

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Doctor of Philosophy in Public Health (Epidemiology)

University of California San Diego, 2021
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Background: Pregravid obesity and abnormal gestational weight gain added to the physiological stresses of pregnancy have been shown to be associated with increased risks of morbidity and mortality for both the infant and mother. However, their effects on placental health are still uncertain. The placenta and the umbilical cord facilitate the interchange of nutrients between the

maternal-fetal dyad. Recent research suggests that the placenta influences the metabolic environment *in utero*, which can in turn affect birth outcomes. Therefore, understanding how obesity affects the health of the placenta is vital.

Methods: This dissertation consists of three studies using data from the University of California San Diego Perinatal Biospecimen Repository Cohort. Study 1 evaluated the effect maternal BMI has on indicators of placental health and assessed GDM as a mediator within the casual pathway. Study 2 assessed the effect gestational weight gain has on indicators of placental health. Study 3 built upon results from the first study by stratifying analyses to determine if the effect of maternal BMI on indicators of placental health were modified by fetal sex. The attenuation of the effect of obese maternal BMI on placental outcomes in the presence of chronic villitis was also evaluated in Study 3.

Results: We found that maternal obesity was significantly associated with increased risk of larger placentas, longer umbilical cords, and chronic villitis (Study 1). Women with excessive gestational weight gain were shown to have higher risks of longer umbilical cords, especially in pregnancies with male fetuses (Study 2). We found that in pregnancies with female fetuses, the risk of chronic villitis is increased in women with obesity while other measurements of abnormal placental health were not at an increased risk. This varied in pregnancies with male fetuses, where the risk of larger placentas and longer umbilical cords were increased in women with maternal obesity, but no increased risk of chronic villitis was reported (Study 3).

Discussion: Maternal BMI and gestational weight gain are associated with indicators of placental health. Each can be used to help identify women at high risk for abnormal placentas and provide the opportunity for increased surveillance and early intervention.

Chapter 1

Introduction

Obesity has become an increasingly troublesome condition in the US. In 2015-2016, the prevalence of obesity was 39.8% and affected about 93.3 million of U.S. adults with approximately 41% of women having obesity.[1,2] The prevalence varies slightly within women as it is lower in women age 20-39 (37%) compared to women age 40-59 (45%).[1,2] Obesity is defined as any person with a body mass index (BMI) $\geq$ 30.0 kg/m^2. [3] It can be further defined into the following three classes: Class 1: 30 to < 35 kg/m^2; Class 2: 35 to < 40 kg/m^2; Class 3: 40 kg/m^2 or higher. The other levels of BMI are: underweight (<18.5 kg/m^2), normal (18.5 to < 25 kg/m^2), and overweight (25.0 to < 30 kg/m^2).[3]

Obesity is a complex chronic condition depending on several factors including genetics, hormones, diets, and environments. Women with obesity face multiple obesity-related conditions including coronary heart disease, T2DM, hypertension, and stroke.[2] Obesity has also been noted to create a state of chronic inflammation within the body.[4-6] The main characteristic of obesity is the accumulation of adipose tissue which is recognized as an active tissue that plays a role in regulating biological processes to include immunity and inflammation.[5,6] This state of metainflammation, or metabolically induced inflammation, varies from an acute pro-inflammatory response because it is triggered usually by metabolites and nutrients instead of an infection.[5,6] Therefore, women with obesity are already at higher risk of poor health outcomes prior to becoming pregnant.

Pregnancy causes the body to be in an altered inflammatory state compared to a non-pregnant body and being overweight or having obesity is thought to exaggerate this process in an adverse way. The physiological stress of pregnancy added to obesity and its interrelated comorbidities has been shown to be associated with increased mortality[7] and morbidity for both the infant and mother.[7,8] Women with obesity are at greater risk of developing complications during pregnancy which include gestational diabetes mellitus (GDM) and preeclampsia.[9,10] These increased risks of complications due to excessive maternal weight have also been shown to be associated with increased prenatal, delivery, and postnatal costs.[11]

Infants of mothers with obesity are at higher risk for negative birth outcomes such as large for gestational age (LGA).[8,12] In addition to higher risk of poor birth outcomes, these infants are also at higher risk for developing obesity, cardiovascular disease and type 2 diabetes later on in adulthood.[12,13] Recent research suggests that the metabolic environment the fetus is exposed to *in utero* plays a crucial role in the development of poor birth outcomes in turn setting the stage for metabolic disorders developing during the infant's lifetime.[13]

In addition to pregravid BMI, the amount of weight gained throughout the pregnancy, known as gestational weight gain (GWG), has been shown to be associated with the increased risks of negative birth outcomes.[14-16] Excessive GWG increases the risk of preeclampsia, cesarean section, and macrosomia,[14,15] and insufficient GWG has been shown to increase risk of preterm birth and cesarean delivery.[16] Weight gain during pregnancy is a normal, expected process due to the development and growth of a fetus, placenta, and amniotic fluid. Other processes also add to the amount of weight gained including increase in the size of breasts and uterus as well as increased volume of blood and fat storage.[17] The Institute of Medicine (IOM) has developed recommendations for GWG during pregnancy that are based on a woman's

pregravid BMI. Women who are in the normal BMI category should strive to gain 25-35 pounds; overweight women, 15-25 pounds; and women with obesity (in all classes), 11-20 pounds.[18] Nonetheless, a recent report from the Center of Disease Control (CDC) states that studies show approximately 50% of women gain more than the suggested amount of weight during pregnancy.[19] These results suggest that unhealthy weight gain during pregnancy is the norm rather than the exception. Besides negative maternal and birth outcomes, an increase risk over a lifetime for cardiovascular and metabolic comorbidities in both the mother and child have also been shown to be associated with excessive GWG.[13,20,21] Specifically, increased risks of pediatric and incident maternal obesity over a lifetime have been shown to be associated with excessive GWG.[20.21]

While there in increasing evidence of the effects that maternal BMI and the amount of weight gained during pregnancy have on birth outcomes and maternal health, the understanding of how obesity and GWG effects the development and function of the placenta throughout pregnancy is currently uncertain. The placenta is the organ that facilitates the interchange of nutrients between the mother-fetal dyad. Placental tissue is developed and orchestrated in the early stages of embryonic development when trophoblasts cells are differentiated around seven days after fertilization. Therefore, within a week of fertilization, the mother's body is already interacting with the developing embryo as the trophoblast cells begin invading the uterine wall. The connection between the placenta and the fetus for the duration of the pregnancy is the umbilical cord.[23] Umbilical cord development begins a few weeks after fertilization during week 3 with the formation of the connecting stalk. By week 7, the umbilical cord is fully formed and functioning.[23] Even though these interactions occurs early on in the pregnancy, placental

health, along with the health of the umbilical cord, is usually assessed after birth once the placenta and cord have been delivered.

A healthy, normally functioning placenta is a vital component to experiencing successful maternal and birth outcomes. A women's pregravid BMI can impact the metabolic environment of the embryo and soon to be fetus along with the placenta early on in the pregnancy, even before the pregnancy is confirmed. The placenta is the interface between the mother and fetus by which nutrients are provided and waste is removed so that placental uptake and transport of nutrients is vital for fetal development. Therefore, understanding how the placenta is altered physically and the role this plays in establishing the intrauterine metabolic environment are of interest. The role obesity plays in the development of the placenta is still unclear, however the understanding of how obesity impacts the placenta during pregnancy is currently being revealed in recent studies. Inflammation in the placental tissue has been noted in pregnant women with obesity as well as GDM which may play a role in creating the adverse intrauterine environment by altering the placenta's ability to regulate nutrients.[24,25] Another study previously found that maternal obesity appears to affect the placenta through the mechanism of chronic villitis, a chronic inflammation infiltrate which involves villi, the functional units of the placenta involved in gas and nutrient exchange.[26]

While studies are continuing to show the adverse effects excessive gestational weight gain has on both maternal and infant outcomes, its effect on placental health is less understood. Results from an epigenetic study have suggested that excessive GWG was correlated with umbilical cord DNA methylation patterns at birth.[27] Another epigenetic study assessing the methylation patterns of placental DNA found that pregravid BMI and gestational weight gain were associated the methylation patterns related to obesity.[28] In that same study, a negative

association between GWG and cord blood levels of an adipokine considered to reduce obesity and insulin resistance was reported where decreased adipokine levels were associated to women with excessive GWG.[29] Interestingly, research has shown that the placenta can adapt when exposed to excessive GWG, but only with female fetuses, by reducing the amount of glucose uptake.[30]

The association between maternal obesity and GWG with GDM is interesting in the discussion of placental health. As mentioned earlier, the risk of GDM is increased in women with obesity prior to conception.[10] A study reported about a 50% increased risk of GDM with increased rates of GWG that appeared to depend more on early weight gain rather than weight gained later in the pregnancy.[31] Based on the interrelationship between the placenta and the intrauterine environment, the role in which GDM might play in the pathway between pregravid BMI and placental outcomes is a valid interest. Evidence indicates that offspring of women with GDM are at higher risk of developing obesity, hypertension and renal disease as well as insulin resistance and type2 diabetes mellitus (T2DM).[32] In the US, the prevalence of GDM can range from 2-10% depending on the population and diagnostic methods used[33] with rising trends paralleling the increasing prevalence of obesity and type 2 diabetes.[33,34] GDM generates an altered intrauterine environment to which the fetus is exposed to during development.[34,35] The increase in the maternal glucose available for fetal absorption creates a state of hyperglycemia. Since maternal insulin does not freely cross the placenta as glucose does, the fetal pancreas must increase production of insulin which in turn creates a state of hyperinsulinemia. Insulin promotes growth and adiposity by acting as a fetal growth hormone. Due to these alterations, fetal metabolic programming of various organs is thought to be modified by epigenetic signals and/or dysregulation of inflammatory pathways.[33-35] which can hinder long-term organ function.

At birth, various indicators can be assessed to determine the health of the placental and the umbilical cord. The weight of the placenta is one such measurement. At term, the average weight of a placenta is approximately 500 grams.[36] The weight, and therefore size, of the placenta provides indication of its health and efficiency during pregnancy. The surface area indicates the amount of space that is available for nutrient and waste interchange to occur. A larger placenta indicates a higher rate of nutrient exchange, whereas a smaller placenta indicates the opposite. High placenta weight has been shown to be associated with poor birth outcomes including low Apgar scores, respiratory distress, neurological abnormalities and neonatal death, whereas low placenta weight was shown to be associated with small body size for the infants later in life and altered hemoglobin levels at birth.[37]

Placenta weight is correlated with newborn birth weight. The ratio of placenta weight to birth weight is typically 1:6.[36] The placenta weight to birth weight ratio is a measurement of the efficiency of the placenta during pregnancy. Overweight and obese women are more likely to have larger placentas which in turn is associated with large for gestational age.[10]

In addition, the length of the umbilical cord and the location by which it is inserted into the placenta are also measureable indicators to determine the health of the pregnancy and increased risks of negative birth outcomes.[38] A longer than normal umbilical cord can become wrapped around the fetus and put the fetus at risk for fetal distress and asphyxia.[38,39] Similarly, knots are associated with longer umbilical cords. If a knot occurs and becomes tightened, the compression can cause fetal distress, asphyxia or even intrauterine fetal death.[38,39] Longer umbilical cords can also complicate delivery due to umbilical cord prolapse which occurs when the umbilical cord lies between the cervix and fetus during delivery.[38,40] Lastly, if the umbilical cord inserts near the edge, or margin, of the placenta rather than the center, it is referred to as

marginal cord insertion. This is associated with intrauterine growth restriction, preterm labor, and fetal distress.[38,41]

Chronic villitis (CV) is a placental disc lesion involving the villous tree. These lesions are caused by macrophages and lymphocytes, white blood cells of the immune system, infiltrating tissue of the placenta.[42] Even though some CV occurs due to a response to an infection, many times the cause of the infiltration of these white blood cells is unknown.[42] These lesions are also referred to as villitis of unknown etiology (VUE) and can be destructive of the villous architecture.[42] If the lesions are low grade, the impact clinically is mild. But, if the extent of tissue involvement is high, then CV has been shown to be associated with intrauterine growth restriction, multiple pregnancy losses, and preterm delivery.[43]

To fully understand the association between pregravid BMI and GWG with placental health, defining possible confounders and adjusting for them in the analyses is a must. Maternal age, parity, race, and ethnicity are all possible confounders that warrant inclusion. In regards to maternal BMI, as they age, women tend to gain weight and therefore have higher BMIs. As previously mentioned, the prevalence of obesity varies among women. It is lower in women age 20-39 at 37% compared to women age 40-59 at 45%.[1] While the effects maternal age has on placental health is less understood, the risks of poor birth outcomes is higher in women > 40 years of age,[44] with 35 years of age being considered "advanced maternal age" and any pregnancy in a women 35 years of age or older considered "high risk". Parity, which is the number of live births a women has had, is also associated with greater weight and BMI. A recent study showed that the mean BMI increased for each additional parity group, (i.e., each additional child) and women on average gained 0.62 (0.58–0.65) BMI units for every birth.[45] As for

placental outcomes, multiparity was shown to be associated with placenta abruption and placental previa.[46]

The prevalence of obesity varies among races and ethnicities. In 2017-2018, non-Hispanic Asian women had a prevalence of obesity at 17.2% which was lower compared to non-Hispanic white women with 39.8%, Hispanic women with 39.8% and non-Hispanic black women with a prevalence of obesity at 56.9%.[47] The prevalence of GDM also varies among races and ethnicities ranging from 5-10% with lower prevalence in non-Hispanic black women and higher prevalence in Asian/Pacific Islander women.[48] One study reported that the risk of GDM increases with increasing maternal BMI for all racial/ethnic groups.[48] While the prevalence of GDM was lower in non-Hispanic black women, the fraction reported to be attributed to maternal BMI was highest at 50.4% compared to 15.1% in Asian/Pacific Islanders, 39.1% among Hispanics, and 41.2% among non-Hispanic white women.[48] In a study looking at prevalence of placental previa among racial and ethnic groups, Asians (OR 1.73, 95% CI 1.53–1.95) and black women (OR 1.43, 95% CI 1.19–1.72) had increased risk of having placental previa compared to white women.[49] Another study did not find an association between race/ethnicity and placental weight in a cohort of very low birthweight infants.[50]

The objectives of this dissertation were to evaluate the effects of maternal exposures, pregravid BMI and gestational weight gain, on various characteristics of the placenta and umbilical cord that indicate the health of these organs during gestation. Each of the studies has a specific aim to provide interrelating results to meet the overall objective. Study 1 estimates the effects that pregravid BMI has on indicators of placenta and umbilical cord health and evaluates the mediating role that GDM has on the causal pathway between maternal BMI and placental weight. Study 2 estimates the effect gestational weight gain has on indicators of placental and

umbilical cord health. Study 3 estimates the effects of pregravid BMI on placental and umbilical cord characteristics and defines the modification of those effects by fetal sex.

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Chapter 2

The effects of pregravid maternal BMI on indicators of placental health

Background: Obesity, along with its serious health consequences, is an increasingly common condition in the United States with over 40% of women having a body mass index in the obese range. The physiological stress of pregnancy along with obesity has been reported to increase poor birth and health outcomes of both the infant and mother. Placental health is influenced by a myriad of maternal factors and the effect pregravid maternal body mass index has on the development and function of the placenta and umbilical cord is still uncertain.

Objective(s): The study estimated the effect maternal body mass index had on indicators of placental health at delivery.

Study Design: This retrospective observational study used data from the University of California San Diego Perinatal Biospecimen Repository Cohort between September 1, 2010 to August 31, 2015. Eligible patients were at least 18 years old with a measured maternal height and weight measured in the first 14 weeks of gestation. Those with pre-existing Type I or Type II diabetes mellitus were excluded. Outcomes of interest were placental weight, umbilical cord length, umbilical cord insertion, and chronic villitis. Poisson regression models were used to estimate the risk ratios of maternal body mass index and placental health measurements.

Results: Within the Perinatal Biospecimen Repository Cohort, 965 patients were identified to meet the study criteria. The average age of the cohort was 31.5 years old (SD, 5.5) with over half of the cohort having either an overweight or obese maternal body mass index. After adjusting for maternal age, ethnicity, parity, fetal sex, and race, women with maternal obesity

had over twice the risk (adjusted risk ratio: 2.10, 95% confidence interval: 1.30, 3.40; p = 0.0026) of having an LGA placental weight compared to women with lower reported maternal body mass index (i.e., underweight/normal, overweight). The risk of large for gestational age umbilical cord length was also doubled (adjusted risk ratio: 2.04, 95% confidence interval: 1.24, 3.35; p=0.0053) in women with maternal obesity compared to women with lower maternal body mass index. The risk of inflammation noted by a chronic villitis diagnosis was also increased in women with maternal obesity, but only in pregnancies with female fetus (adjusted risk ratio: 1.79, 95% confidence interval: 1.05, 3.04; p=0.0313). A proportion (4.6%) of the increased risk of large for gestational age placental weight in women with maternal obesity was shown to be mediated by gestational diabetes.

Conclusion(s): Women with maternal obesity studied in routine clinical practice at an academic center were shown to have higher risks of larger placentas, longer umbilical cords and chronic villitis.

The effects of maternal BMI on indicators of placental health

Background

Within the U.S., obesity has become an increasingly common condition. Approximately 41% of women have obesity, with a slightly lower prevalence in women 20-39 years of age (37%) compared to 40-59 years of age (45%).[1,2] Obesity increases the risk of many conditions, including coronary heart disease, Type 2 diabetes mellitus (T2DM), hypertension, and stroke, and is also associated with chronicinflammation.[2] The physiological stress of pregnancy added to obesity and its related comorbidities has been shown to increase morbidity and mortality for both the infant and mother.[3,4] Pregnant women with obesity are at greater risk of developing

gestational diabetes mellitus (GDM) and preeclampsia[5,6] with variability noted among different races and ethnicities.[7] The increased risk of GDM, specifically, has been reported up to 2-fold in women with obesity. [6-8]

Infants born to mothers who have obesity are at higher risk for negative birth outcomes, such as large for gestational age (LGA),[5] and they are also more likely to develop obesity, cardiovascular disease, and T2DM later in life.[9] However, it is not known whether an elevated pregravid BMI affects the development and function of the placenta. The placenta, along with the umbilical cord, facilitates the interchange of nutrients and waste products between the maternal-fetal dyad. The development of the placenta begins at implantation, and precedes early stages of embryonic development; therefore, the mother's health at conception is important to placental development and the entire course of pregnancy. Recent research suggests that the placenta influences the metabolic environment *in utero,* which can in turn affect birth outcomes, as well as set the stage for development of metabolic disorders during the infant's lifetime.[10] Therefore, understanding how obesity affects the health of the placenta is important. Recent studies have reported that maternal obesity and GDM were associated with inflammation in placental tissue examined after birth.[11,12] We have previously found that maternal pre-pregnancy obesity is associated with chronic villitis, a chronic inflammatory infiltrate that involves the chorionic villi.[13] Interestingly, in the setting of maternal obesity, villitis was more common in the placentas of female offspring.[13]

Here, we hypothesize that chronic villitis, as well as other cellular and molecular alterations in the placenta induced by maternal obesity and GDM, play a role in establishing an adverse intrauterine environment for the fetus by altering the placenta's ability to regulate nutrients. We investigated the associations between pregravid BMI and placental function in a

study cohort of pregnant women with linked demographic and clinical data, as well as placental pathology data, allowing adjustment for clinical characteristics.

Methods

This retrospective cohort study utilized data from the University of California San Diego (UCSD) Perinatal Biospecimen Repository Cohort[14] over a 5-year study period, from September 1, 2010 to August 31, 2015. In the Perinatal Biospecimen Repository Cohort, both low- and high- risk pregnant patients were enrolled to obtain clinical data and multiple biospecimens, including placental tissue. Women who were at least 18 years old at time of delivery and had a measured maternal height and weight in the first 14 weeks of gestation were included in the study (n=1,128). Women with pre-existing Type 1 diabetes mellitus (T1DM) or T2DM, were excluded from the study (n=163). Maternal demographic, anthropometric, and obstetric data, as well as neonatal outcome data, were abstracted from the electronic health record (EHR) data source housed in EPIC (Epic Systems Corporation, Verona, WI).

This study was approved by the Human Research Protections Program of the University of California San Diego; all participants provided written informed consent prior to participation in the Perinatal Biospecimen Repository Cohort.

Outcome Assessment

The four pathologic outcomes assessed during the study were placental weight; umbilical cord length; umbilical cord insertion; and a histological finding of chronic villitis (CV).[15] Each of these outcomes was assessed after delivery. Placental gross and microscopic examination and preparation of slides for histology were supervised by the same Board-Certified Anatomic Pathologist with fellowship training in Perinatal Pathology (M. Parast).

For placental weight and umbilical cord length, gestational age-adjusted percentiles based on published nomograms were calculated, and then the percentiles were used to categorize the placental weights and umbilical cord lengths into three levels: small for gestational age (SGA), appropriate for gestational age (AGA), and large for gestational age (LGA).

Umbilical cord insertion was dichotomized into 'Normal' (for central cord insertions) and 'Abnormal' (for marginal, velamentous, or furcate cord insertions) based on the location of the insertion point of the umbilical cord to the placenta. Finally, a diagnosis of CV was categorized as either present or absent by the reviewing pathologist, regardless of the grade of the lesion.

Covariate Assessment

All relevant covariates were determined based on the use of a directed acyclic graph (DAG) as an aid in determining confounders and mediators.[16] The primary 'exposure' of interest for this study was maternal BMI. Maternal BMI was calculated using a maternal weight and height recorded at less than 14 weeks gestation, with the earliest available measurement used for analysis. Maternal BMI was categorized based into four groups: underweight (<18.5), normal (18.5 to < 25), overweight (25.0 to < 30), and obese ($\geq$ 30.0).[17] Possible confounders included in our analysis were maternal age, parity, gestational age (GA) at delivery, fetal sex, GDM status, and mother's self-reported race and ethnicity. Maternal age at the time of conception was calculated using the mother's birthdate and date of conception based on estimated gestational age from the earliest recorded date for prenatal care. Parity, i.e., number of deliveries after 20 weeks' gestation, was coded based on self-reported data and remained an ordinal variable in analyses. Using EPIC, GA at delivery was estimated from the date of the first day of the last menstrual period (if available) together with fetal biometrical measurements taken during

ultrasounds. GA was measured in weeks and maintained as a continuous variable during analyses. Fetal sex was a dichotomous variable noted as either 'Male' or 'Female'. Maternal ethnicity was categorized as 'Hispanic' or 'Non-Hispanic', and race categorized as either 'White', 'Black', 'Asian', or 'Other' both of which were based on self-reported data from EPIC.

For this study, GDM status was determined through review of laboratory results noted within EPIC records and applying guidelines based on American Diabetes Association recommendations. Glucose tolerance test (GTT) results performed during the second trimester were reviewed and a woman was categorized as having GDM if she had a value ≥135 mmol/L from a 1 hour GTT, and failed at least two of the steps of a three hour GTT (fasting ≥95 mmol/L, 1-hour ≥180 mmol/L, 2-hour ≥155 mmol/L, or 3-hour ≥140 mmol/L).[18]

Statistical Analysis

Underweight and normal BMI categories were combined due to low numbers of subjects in the underweight BMI level. Descriptive statistics for continuous and categorical variables were presented by each of the outcomes. We performed χ^2 tests to assess the association between maternal BMI and maternal characteristics. The least-square means were calculated for placental weight and umbilical cord length among each of the BMI categories and plotted with tests for linear trend performed. To assess the linear association between maternal BMI and gestational-age adjusted placental weight and umbilical cord length, generalized linear models were constructed to estimate β coefficients. Poisson regression models were used to estimate the risk ratios of the effect between pregravid obesity and each of the placental health outcomes. For these models, both placental weight and umbilical cord length were dichotomized into LGA vs. non-LGA. All covariates were included in the full models. GDM was treated as a mediator in

these analyses and therefore not included in the primary model. Maternal BMI was also assessed as continuous in Poisson regression models to estimate the effect size of the association between maternal BMI and abnormal UC insertion and CV.

Based on the DAG, GDM was hypothesized to mediate the effect between maternal BMI and gestational age-adjusted LGA placental weight. Once the exposure-mediator association was determined to be present and assumptions were met, casual mediation analysis was performed.[19] Maternal age, parity, infant sex, ethnicity, and race were the confounders added to the models to reduce bias. The total effect between maternal BMI and LGA placental weight mediated by GDM was reported. Data were analyzed using SAS, version 9.4 for Windows (SAS Institute Inc., Cary, NC).

Results

Table 1 shows the descriptive characteristics of the 965 patients identified from the Perinatal Biospecimen Repository Cohort that met all study criteria. Descriptive characteristics of the initial cohort of patients considered for inclusion (n=1,391) are shown in Table 4 in the Appendix. The average age of the study cohort was 31.5 years old (SD 5.5) with 66% of the cohort greater than 30 years of age. The mean gestational age at delivery within the cohort was 38.3 weeks (SD 2.9). Approximately half (52%) of the cohort had a BMI that was in the overweight or obese categories. The women represented in the study cohort were more likely to be non-Hispanic than Hispanic and white than other races.

LGA placentas were more likely from pregnancies affected by maternal obesity compared to non-LGA placentas (LGA, 46.1 %; AGA, 29.4%; SGA, 17.8%) (Table 1). An opposite trend was seen in SGA placentas with a higher fraction of SGA placentas from

underweight/normal maternal BMI pregnancies (62.4%) compared to both AGA (47.5%) and LGA (39.3%) placentas.

Maternal BMI was shown to be significantly associated with gestational age-adjusted UC length (Table 1). A higher fraction of LGA UC lengths were from pregnancies affected by obesity (41.9%) compared to shorter UC lengths (AGA, 29.8%, SGA, 18.4%) whereas SGA UC lengths were more likely from pregnancies with underweight/normal maternal BMI (56.1%) compared to longer UC lengths (AGA, 48.0%; LGA, 38.4%) ($p<0.001$). Women with a CV diagnosis were more likely to have had maternal obesity (39.3%) and less likely to have had underweight/normal maternal BMI (37.0%) compared to women without a CV diagnosis (obesity, 27.7%; underweight/normal, 50.3%)($p = 0.007$) (Table 1). Among mothers with maternal obesity, the proportion of pregnancies with female fetuses which had CV diagnosis was 27% while the proportion of pregnancies of male fetuses with a CV diagnosis was 20%, although this was not significant. On the other hand, the distribution between female and male placentas diagnosed with CV among mothers without obesity was the same (15.5%).

Figure 1 displays the mean percentiles and 95% CI of gestational age-adjusted placental weight and umbilical cord length by maternal BMI. Percentiles of placental weight ($p<0.0001$, linear trend) and umbilical cord length ($p = 0.012$, linear trend) increased as maternal BMI increased.

As shown in Table 2, there were significant associations between maternal obesity and LGA placental weight, LGA UC length, and CV both before and after adjustment for potential confounders. Women with maternal obesity had over twice the risk (aRR: 2.10, 95% CI: 1.30, 3.40; $p = 0.0026$) of having an LGA placental weight compared to women with lower reported

maternal BMI (i.e., underweight/normal, overweight. In addition, we found a significant positive association between maternal BMI and gestational age-adjusted placenta weight ($\beta = 0.573$, $p < 0.0001$). The relative risk for LGA umbilical cord length was significantly increased in women with obesity, with a doubling of risk (aRR: 2.04, 95% CI: 1.24, 3.35; p=0.0053) compared to women with lower maternal BMI after model adjustment. Gestational age-adjusted umbilical cord length had a significant positive association with maternal BMI ($\beta = 0.324$, $p = 0.008$). The risk of CV in women with obesity was increased by 51% (aRR: 1.51, 95% CI: 1.04, 2.19; p=0.0294) compared to women with underweight/ normal or overweight maternal BMI. When maternal BMI was assessed as a continuous variable, it was positively associated with risk of CV (aRR: 1.03, 95% CI: 1.01, 1.05).

Table 3 shows the results of the causal mediation analysis. The total effect of maternal obesity on LGA placental weight was 2.10; 95% CI: 1.3, 3.4. The effect of maternal obesity on LGA placental weight not mediated by GDM (i.e., the direct effect) was 1.91; 95% CI 1.19, 3.02. Finally, the indirect effect, the effect maternal obesity has on LGA placental weight mediated by GDM, was 1.10; 95% CI 1.01, 1.22. The proportion of the effect of maternal BMI on gestational age-adjusted placental weight mediated by GDM was 4.6%, suggesting that GDM is a partial mediator of the effect of maternal obesity on LGA placental weight.

Discussion

This study assessed the effect of maternal BMI on placental weight, UC length, UC insertion, and CV in a cohort of women treated within an academic care center. In agreement with our hypotheses, there was an increased risk of abnormal placental findings associated with maternal BMI. We found that maternal obesity was significantly associated with increased risk of larger placentas, longer UC cords, and inflammation as indicated by a diagnosis of CV.

The magnitudes of the observed effects are clinically relevant. Maternal BMI was positively associated with increased risk of high placental weight and long UC length even accounting for gestational age, suggesting that a high pregravid BMI increased the risk of developing large-for-gestational age placentas and umbilical cords. This association remained after adjustment for maternal age, parity, fetal sex, race, and ethnicity. Further analyses indicated that women with pregravid obesity had twice the risk of developing an LGA placenta and a longer than normal umbilical cord during pregnancy as compared to women with a pregravid BMI below 30. Leon-Garcia et al (2015) found similar associations between maternal obesity and large placental weight, concluding that women with pregravid obesity had more than double the increased risk for large placentas.[13] While this earlier study used subjects from the same Perinatal Repository, we further adjusted for confounding by demographic characteristics known to be associated with maternal BMI and birth outcomes, such as maternal race and ethnicity, which might explain why our effect size was somewhat less than that reported in the previous study.[13]

Recent research on the effect of maternal BMI on umbilical cord length is scarce, but alteration in gene expression associated with inflammation, insulin resistance, and epigenetic programming through DNA methylation within the placenta and cord blood has been linked to maternal obesity.[20,21] Our study suggests that women with pregravid obesity have twice the risk of developing a longer than normal umbilical cord, which has been shown to be associated with delivery complications as well as cord prolapse, torsion, and true knot entanglement.[22]

Women with pregravid obesity were reported to have a 50% increase in the risk of CV, with preliminary findings suggesting the risk might vary by fetal sex. An increased risk of CV indicates an increased risk of inflammation in the placental tissue. These findings are supported

by previous pathology studies that reported that maternal obesity was associated with increased inflammation in placental tissues, and that the increase was more pronounced in pregnancies with a female fetus.[22] The clinical significance of this increased risk of CV remains uncertain, however. The majority of these lesions were low-grade and likely secondary to non-infectious villitis (also known as villitis of unknown etiology/VUE).[23] When severe, VUE can cause fetal growth restriction, among other perinatal complications.[24] In the setting of maternal obesity, the effect of CV on fetal growth remains to be elucidated.

Based on causal mediation analysis, it appears that GDM may play a small role in mediating the effects of maternal obesity on placental weight (4.6%). We hypothesized that GDM would be a mediator, but the magnitude was smaller than expected. Women with pregravid obesity are at a higher risk of developing GDM during pregnancy,[25, 26] which is supported by the findings of our mediation analysis. The unexpectedly small effect might be due to the definition of GDM that was used. Both GDMA1 (i.e., GDM managed with diet alone) and GDMA2 (i.e. patients receiving medication in order to control GDM during their pregnancy) were included in this study. Also, while patients with a diagnosis in EPIC of either T1DM or T2DM were removed from the study, women with T2DM that were not diagnosed prior to pregnancy could have been categorized as GDM from the lab results reported in EPIC. The inclusion of GDMA1 could have dampened the mediation effect. Future studies should consider specifically assessing the mediating effect of GDMA2, to determine if severity of GDM during pregnancy is a contributing factor to this mediating effect.

There are several limitations to our study. Our cohort represents a group of women who were more likely to receive prenatal care and deliver at a regional academic care center, which might limit generalizability. Women without a maternal weight measurement during the first 14

weeks of gestation were excluded from this study which was the main criteria that removed patients from the study and represented 17% of the initial cohort. Based on the descriptive statistics of the initial cohort considered for analysis prior to applying the inclusion and exclusion criteria, the final study cohort had fewer Hispanic patients than the initial cohort as well as less patients with an "Other" race categorization. Due to the lack of early data points regarding weight, these women might represent patients with minimal prenatal care, which has been linked to poor birth and maternal outcomes at delivery.

Nevertheless, this study also has numerous strengths. Our sample size was large, allowing for stratification by variables of interest, including BMI, ethnicity, race, and GDM. The ability to abstract information from both the Perinatal Repository and medical records in EPIC provided the opportunity to create a robust data set that allowed for the incorporation of various potential confounders, both demographic and clinical. In addition, all placentas were evaluated in a blinded manner by a single pathologist specializing in placental assessment, providing consistent results and avoiding interobserver variability.

Our findings show that maternal BMI is associated with the health of the placenta. Women in their child-bearing years who are considering becoming pregnant may benefit from decreasing their weight/BMI with regard to reducing the risk of poor placental health outcomes. Our findings suggest that even small decreases in weight may have a positive impact on placental phenotypes, especially in women with obesity. Furthermore, maternal BMI can be used to help identify women at high risk for abnormal placentas, which could lead to poor birth outcomes and provide the opportunity for increased surveillance and early intervention.

Acknowledgments

Chapter 2, in full, is currently being prepared for submission for publication of the material. Rush, Toni M; Laurent, Louise C; Quintana, Penelope J; Jain, Sonia; Thompson, Caroline A; Parast, Mana; Chambers, Christina D. Toni Rush was the primary investigator and author of this material.

Table 2-1 Patient Characteristics by Placental Outcomes, 2010-2015

	Overall		Placental Weight				Cord Length				Abnormal Cord Insertion			Chronic Villitis		
			SGA	AGA	LGA		SGA	AGA	LGA		No	Yes		No	Yes	
	(n=965)		(n=101)	(n=775)	(n=89)		(n=98)	(n=781)	(n=86)		(n=841)	(n=124)		(n=792)	(n=173)	
	N (%)		N (%)	N (%)	N(%)	p-value	N(%)	N(%)	N(%)	p-value	N(%)	N(%)	p-value	N(%)	N(%)	p-value
	28.0 mean, 8.2 SD					**0.0002**				**0.016**			0.462			**0.007**
rmal	463	(48.0)	63(62.4)	365(47.1)	35(39.3)		55(56.1)	375(48.0)	33(38.4)		399(47.4)	64(51.6)		399(50.3)	64(37.0)	
	215	(22.3)	20(19.8)	182(23.5)	13(14.6)		25(25.5)	173(22.2)	17(19.8)		186(22.2)	29(23.4)		174(22.0)	41(23.7)	
	287	(29.7)	18(17.8)	228(29.4)	41(46.1)		18(18.4)	233(29.8)	36(41.9)		256(30.4)	31(25.0)		219(27.7)	68(39.3)	
ears)	31.5 mean, 5.5 SD					0.754				0.176			0.205			0.583
	116	(12.0)	9(8.9)	93(12.0)	14(15.7)		10(10.2)	102(13.1)	4(4.7)		102(12.1)	14(11.3)		96(12.1)	20(11.6)	
	229	(23.7)	26(25.7)	179(23.1)	24(27.0)		18(18.4)	186(23.8)	25(29.1)		196(23.3)	33(26.6)		44(25.4)	44(25.4)	
	324	(33.6)	33(32.7)	264(34.1)	27(30.3)		35(35.7)	262(33.5)	27(31.4)		292(34.7)	32(25.8)		63(36.4)	63(36.4)	
	296	(30.7)	33(32.7)	239(30.8)	24(26.9)		35(35.7)	231(29.6)	30(34.9)		251(29.9)	45(36.3)		46(26.6)	46(26.6)	
	1.8 mean, 0.9 SD					**0.048**				0.257			**0.009**			0.218
	452	(46.8)	56(55.4)	366(47.2)	30(33.7)		55(56.1)	366(46.9)	31(36.0)		382(45.4)	70(56.5)		383(48.4)	69(39.9)	
	307	(31.8)	31(30.7)	244(31.5)	32(36.0)		27(27.6)	248(31.8)	32(37.2)		275(32.7)	32(25.8)		247(31.2)	60(34.7)	
	128	(13.3)	8(7.9)	101(13.0)	19(21.3)		10(10.2)	103(13.2)	15(17.4)		109(13.0)	19(15.3)		100(12.6)	28(16.2)	
	78	(8.1)	6(5.9)	64(8.3)	8(9.0)		6(6.1)	64(8.2)	8(9.3)		75(8.9)	3(2.4)		62(7.8)	16(9.2)	
						0.191				0.304			0.159			0.966
	696	(72.1)	67(66.3)	569(73.4)	60(67.4)		77(78.6)	559(71.6)	60(69.8)		600(71.3)	96(77.4)		571(72.1)	125(72.2)	
	269	(27.9)	34(33.7)	206(26.6)	29(32.6)		21(21.4)	222(28.4)	26(30.2)		241(28.7)	28(22.6)		221(27.9)	48(27.7)	
						0.879				0.210			0.530			0.409
	496	(51.4)	52(51.5)	396(51.1)	48(53.9)		49(50.0)	395(50.6)	52(60.5)		429(51.0)	67(54.0)		412(52.0)	84(48.6)	
	469	(48.6)	49(48.5)	379(48.9)	41(46.1)		49(50.0)	386(49.4)	34(39.5)		412(49.0)	57(46.0)		380(48.0)	89(51.4)	
						0.003				0.752			**0.041**			**0.009**
	613	(62.4)	79(78.2)	484(62.5)	50(56.2)		59(60.2)	500(64.0)	54(62.8)		524(62.3)	89(71.8)		518(65.4)	78(45.0)	
	352	(37.6)	22(21.8)	291(37.5)	39(43.8)		39(39.8)	281(36.0)	32(37.2)		317(37.7)	35(28.2)		274(34.6)	95(54.9)	
						0.113				0.649			0.068			0.571
	524	(54.3)	61(60.4)	409(52.8)	54(60.1)		48(49.0)	426(54.5)	50(58.1)		447(53.2)	77(62.1)		434(54.8)	90(52.0)	
	56	(5.8)	8(7.9)	45(5.8)	3(3.4)		4(4.1)	49(6.3)	3(3.5)		52(6.2)	4(3.2)		47(5.9)	57(32.9)	
	108	(11.1)	14(13.9)	88(11.4)	6(6.7)		13(13.3)	84(10.8)	11(12.8)		101(12.0)	7(5.7)		91(11.5)	9(5.2)	
	277	(28.7)	18(17.8)	233(30.1)	26(29.2)		33(33.7)	222(28.4)	22(25.6)		241(28.7)	36(29.0)		220(27.8)	17(9.8)	

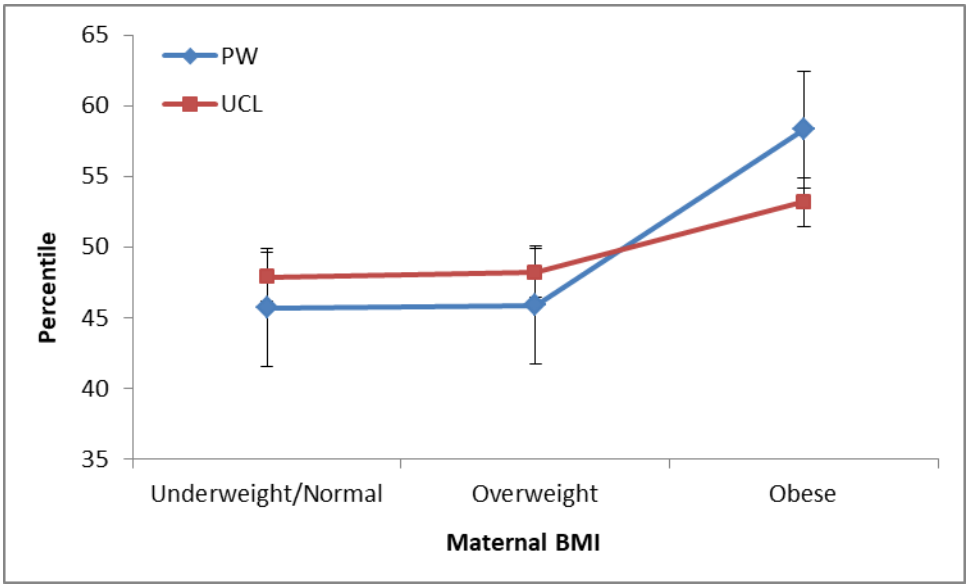

Figure 2-1 Gestational age-adjusted percentile of placental weight (PW) ($p<0.0001$) and umbilical cord length (UCL) ($p=0.012$) by maternal BMI. NOTE: p-values represented linear test for trend; y-axis range does not begin at zero.

Table 2-2 Risk Ratios of Maternal BMI effect on Placental Health Outcomes, 2010-2015 (n=965)

	Obesity*					
	Unadjusted RR			Adjusted RR		
Placental Health Outcomes**	**RR**	**95%CI**	**p-value**	**aRR**	**95% CI**	**p-value**
LGA Placental Weight	2.19	(1.41, 3.40)	**0.0005**	2.10	(1.30, 3.40)	**0.0026**
LGA UC Length	1.80	(1.15, 2.83)	**0.0108**	2.04	(1.24, 3.35)	**0.0053**
Abnormal UC Insertion	1.31	(0.85, 2.02)	0.2172	1.06	(0.67, 1.69)	0.8010
Chronic Villitis	1.69	(1.20, 2.39)	**0.0025**	1.51	(1.04, 2.19)	**0.0294**

Model adjusted for maternal age, parity, fetal sex, ethnicity, race. Abbreviations: BMI – body mass index, RR – relative risk, aRR – adjusted relative risk, CI – confidence interval, LGA – large for gestational age, UC – umbilical cord; * Reference Non-Obese maternal BMI, ** Reference Non-LGA Placental Weight, Non-LGA UC Length, Normal UC Insertion, No Villitis

Table 2-3 Effect decomposition of the influence of maternal BMI and gestational diabetes as a mediator on gestational age-adjusted LGA placental weight (n=965)

	Total Effect[a]		Direct Effect[b]		Indirect Effect[c]		Proportion-Mediated[d]
Mediator	aRR	95%CI	aRR	95%CI	aRR	95% CI	
Gestational Diabetes	2.10	(1.30, 3.40)	1.91	(1.19, 1.02)	1.10	(1.01, 1.22)	4.6%

Model adjusted for maternal age, parity, fetal sex, ethnicity, race

[a]Effect of maternal BMI on LGA placental weight

[b]Effect of maternal BMI on LGA placental weight that is not mediated by gestational diabetes

[c]Effect of maternal BMI on LGA placental weight mediated by gestational diabetes

[d]Proportion of effect of maternal BMI on LGA placental weight mediated by gestational diabetes

Appendix

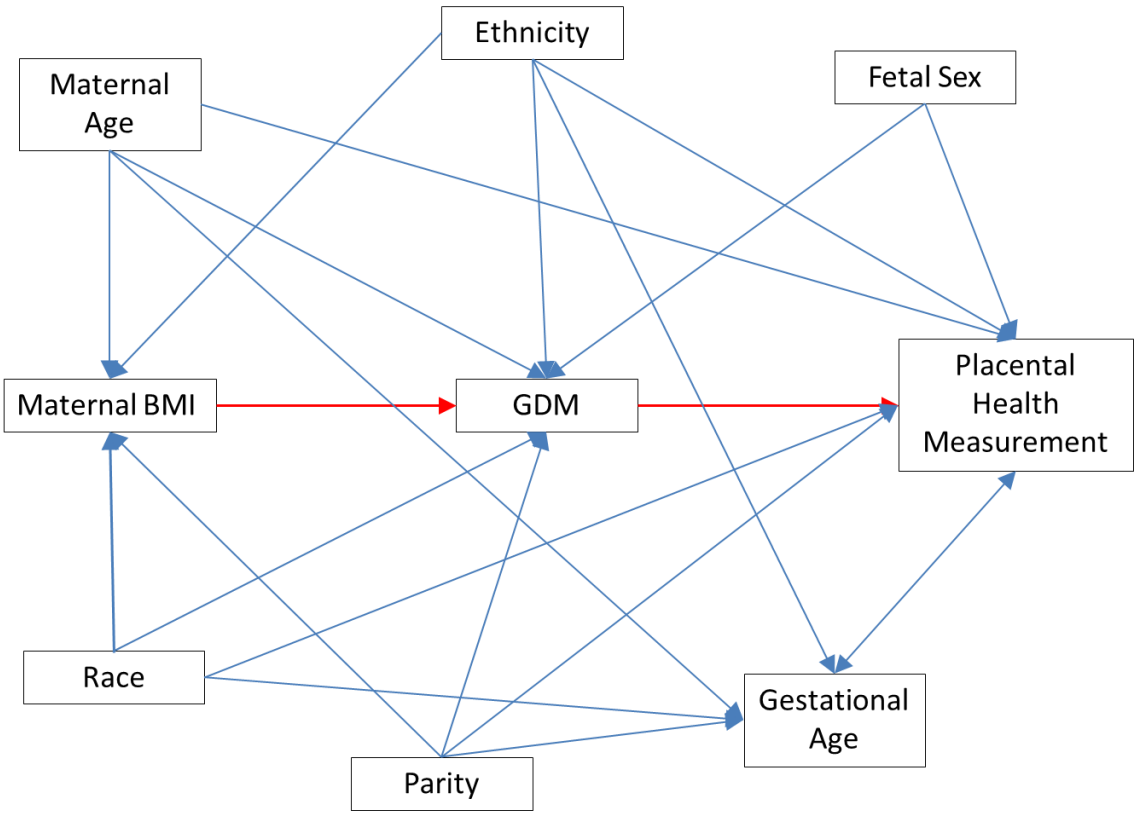

Figure 2-2. Directed acyclic graph used to determine confounders and mediators

Table 2-4 Descriptive Characteristics of Initial and Final Study Cohorts

Characteristics	Initial Cohort (n=1,391) N (%)	Final Cohort* (n=965) N (%)
Maternal BMI	29.0 mean, 8.2 SD	28.0 mean, 8.2 SD
Underweight/Normal	567 (40.7)	463 (48.0)
Overweight	324 (23.3)	215 (22.3)
Obese	500 (36.0)	287 (29.7)
Maternal age (years)	31.2 mean, 5.7 SD	31.5 mean, 5.5 SD
18-24	191 (13.7)	116 (12.0)
25-29	351 (25.2)	229 (23.7)
30-34	440 (31.6)	324 (33.6)
35+	409 (29.4)	296 (30.7)
Parity	1.8 mean, 0.9 SD	1.8 mean, 0.9 SD
0	645 (46.4)	452 (46.8)
1	405 (29.1)	307 (31.8)
2	201 (14.5)	128 (13.3)
3+	140 (10.1)	78 (8.1)
GDM		
No	1062 (76.3)	696 (72.1)
Yes	329 (23.7)	269 (27.9)
Fetal Sex		
Male	707 (50.8)	496 (51.4)
Female	684 (48.2)	469 (48.6)
Ethnicity		
Non-Hispanic	766 (55.1)	613 (62.4)
Hispanic	625 (44.9)	352 (37.6)
Race		
White	670 (48.2)	524 (54.3)
Black	79 (5.7)	56 (5.8)
Asian	133 (9.6)	108 (11.1)
Other/Mixed	509 (36.6)	277 (28.7)

*Final cohort once inclusion and exclusion criteria were applied.

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Chapter 3

Assessing the effects of gestational weight gain on multiple measurements of placental health

Background: The amount of weight women gain during pregnancy can impact maternal and infant birth outcomes. The effect that gestational weight gain has on placental and umbilical cord health is still poorly understood.

Objective(s): This study aimed to estimate the effect gestational weight gain has on indicators of placental health and to determine if those effects vary by fetal sex.

Study Design: This retrospective observational cohort study used data from the University of California San Diego Perinatal Biospecimen Repository Cohort collected from patients between September 1, 2010 to August 31, 2015. Eligible patients were at least 18 years old with a measured maternal height and weight in the first 14 weeks of gestation as well as a documented maternal weight within one month of delivery. Those with pre-existing Type I or Type II diabetes mellitus were excluded from the study. Outcomes of interest included placental weight, umbilical cord length, umbilical cord insertion, and chronic villitis. All analyses were stratified by fetal sex. Poisson regression models were used to estimate the risk ratios of gestational weight gain and placental health measurements.

Results: Within the Perinatal Biospecimen Repository Cohort, 957 patients were identified to meet the study criteria. The average age of the cohort was 28.0 years old (SD 8.2) with two-thirds of the cohort having abnormal gestational weight gain defined by either insufficient or

excessive, regardless of the fetal sex. In pregnancies with male fetuses, patients who experienced excessive weight gain had over three times the risk (aRR: 3.05, 95% CI: 1.45, 6.41; p = 0.0033) of having an LGA umbilical cord compared to women with normal gestational weight gain. In pregnancies with female fetuses, the magnitude of increased risk of LGA umbilical cord length among women with excessive gestational weight gain was smaller, and not significant.. The remaining outcomes were not significantly different for either fetal sex.

Conclusion(s): Women with excessive gestational weight gain studied in routine clinical practice at an academic center were shown to have higher risks of longer umbilical cords, especially in pregnancies with male fetuses.

Assessing the effect of gestational weight gain on placental health measurements

Background

There is increasing evidence that the amount of weight gain during pregnancy can impact the health of the mother and infant. The Institute of Medicine (IOM) has developed guidelines for gestational weight gain (GWG) based on a woman's pregravid BMI[1]. The IOM recommends that women who are in the normal BMI category aim to gain 25-35 pounds; overweight women, 15-25 pounds; and women with obesity (in all classes), 11-20 pounds[1]. Even with these guidelines, recent data show that only about 32% of women gain the recommended amount of weight, with 21% gaining too little and 48% gaining too much[2,3]. These results suggest that unhealthy weight gain during pregnancy is the norm rather than the exception. Both insufficient and excessive GWG have been associated with poor birth outcomes for both the mother and the

infant. Increased risks of preeclampsia, cesarean section, and macrosomia have been shown to be associated with increased GWG[4]. On the other hand, insufficient GWG has been shown to increase risk of preterm birth and cesarean delivery [5]. In addition to these negative maternal and birth outcomes, an increased lifetime risk of cardiovascular and metabolic comorbidities in both the mother and child, as well as pediatric and incident maternal obesity, have been shown to be associated with excessive GWG[1,6,7].

While studies are continuing to report adverse effects of insufficient and excessive GWG on both maternal and infant outcomes, their effect on placental health is not well understood. The placenta, along with the umbilical cord, is the interface between the mother and fetus by which nutrients are provided and waste is removed; therefore, placental uptake and transport of nutrients is vital for fetal development[8,9]. Due to the evidence that maternal obesity as well as GWG are associated with increased fetal growth, understanding how the placenta and umbilical cord are altered physically and the role they play in establishing the intrauterine metabolic environment are of interest. Results from an epigenetic study have suggested that excessive GWG was correlated with altered DNA methylation patterns in umbilical cord tissue at birth[10]. Another study reported a negative association between GWG and cord blood levels of an adipokine considered to reduce obesity and insulin resistance where decreased adipokine levels were reported in women with excessive GWG[11].

A healthy functioning placenta and umbilical cord are vital components to experiencing successful maternal and birth outcomes. Studies have shown that the placenta can adapt when exposed to excessive GWG by reducing the amount of glucose uptake, and that this is more efficiently done by placentas of female offspring[12]. This study aims to quantify the sexually dimorphic effect of GWG on placental health, both macroscopic and microscopic, within a study

cohort of women with available demographic and placental pathologic data, to adjust for clinical and pathologic characteristics. Our hypothesis is that, compared to normal GWG, excessive and insufficient GWG will affect the health of the placenta and those effects vary by the sex of the fetus.

Methods

We conducted a retrospective cohort study utilizing clinical data and placental tissue samples from patients enrolled in the UCSD Perinatal Biospecimen Repository Cohort[13] over a 5-year study period from September 1, 2010 to August 31, 2015. Both low- and high- risk pregnant patients are consented and enrolled in the Perinatal Biospecimen Repository Cohort. Clinical data and multiple biospecimens, including placental tissue, are collected and stored for patients enrolled in the cohort. The subjects for this current study were selected from the total available subjects enrolled into the UCSD Perinatal Biospecimen Repository Cohort. Patients who delivered between September 2010 and August 2015, were at least 18 years old at time of delivery, and had a measured maternal weight during the first 14 weeks of gestation and within one month prior to delivery were included in the study. Patients without the two required measurements for maternal weight and those with T1DM and T2DM were excluded from the study. The Perinatal Biospecimen Repository Cohort contains 1,378 subjects who delivered between September 2010 and August 2015. After excluding 250 subjects who did not have a maternal weight recorded during the first 14 weeks of gestation, 8 subjects without a recorded maternal weight within 1 month of delivery, 37 subjects with T1DM, and 139 subjects with T2DM, the remaining 957 subjects formed the basis for the study analysis. Once the final cohort that met the inclusion and exclusion criteria was identified, maternal demographic, anthropometric, and obstetric data were abstracted from the electronic health record data source

housed in EPIC (Epic Systems Corporation, Verona, WI). The repository includes patients' medical record number (MRN), which allows the patient to be linked to their EPIC records. Along with the MRN, other identifying characteristics, such as birthdate of mother and birthdate of infant, were used to verify the patient's identity prior to abstraction. Once the patient was verified, abstraction of the additional data was performed to create a final data set.

This study was approved by the Human Research Protections Program of the University of California San Diego; all participants provided written informed consent prior to participation.

Outcome Assessment

Outcomes of interest included placental characteristics known to be markers of the overall health of the placenta during gestation. The three pathologic outcomes assessed during the study were placental weight, umbilical cord (UC) length, and a histological finding of chronic villitis (CV)[14]. Each of these outcomes was assessed after delivery. Gross and histological placental examinations were performed for those with a clinical indication for placental exam through the UCSD Pathology Department. Those without such indications were processed by the research histology core. In either case, placental gross and microscopic examination and preparation of slides for histology were supervised by the same Board-certified Anatomic Pathologist with fellowship training in Perinatal Pathology (M. Parast).

While the placenta and umbilical cord are established during early gestation, they change dramatically in size across pregnancy. Therefore, for placental weight and UC length, gestational age-adjusted percentiles based on published nomograms were calculated[15] and then the classified into three levels: small for gestational age (SGA), appropriate for gestational age (AGA), and large for gestational age (LGA). Umbilical cord insertion was dichotomized into

'Normal' (for central cord insertions) and 'Abnormal' (for marginal, velamentous, or furcate cord insertions) based on the location of the insertion point of the umbilical cord into the placental disc. Lastly, a diagnosis of CV was categorized as either 'Present' or 'Absent' by the reviewing pathologist, regardless of the grade of the lesion.

Exposures and Covariates

GWG was the primary exposure of interest for this study. GWG was calculated by subtracting the earliest maternal weight recorded at less than 14 weeks gestation from the latest maternal weight recorded within 1 month of delivery. GWG was categorized into 3 groups based on pregravid BMI and overall weight: insufficient, adequate, and excessive. Insufficient GWG is defined as total weight gain below the respective range established for each level of maternal BMI, while excessive GWG is defined as total weight gain above the established range. Normal GWG is defined as total weight gain that falls within the established range for each level of maternal BMI. The recommended range of total GWG varies depending on pregravid BMI. Women who were recorded with an initial underweight BMI have a recommended range between 28 – 40 pounds, normal BMI between 25-35 pounds, overweight between 15-25 pounds, and obese (in all classes) between 11-20 pounds[1].

All relevant covariates were determined based on review of the literature and clinical input. Covariates we considered as potential confounders in our analysis included maternal age, parity, maternal BMI, gestational age (GA), diagnosis of gestational diabetes mellitus (GDM), and patient race and ethnicity. All analyses were stratified by fetal sex, noted as either 'Male' or 'Female'. Maternal age at conception was calculated using the mother's birthdate and the gestational age at the earliest recorded date for prenatal care and included as a continuous

variable for adjustment. Parity, i.e., the total count of deliveries after 20 weeks of gestation at the time of study enrollment, was self-reported and included as an ordinal variable for adjustment. Pregravid maternal BMI was categorized based into four groups: underweight (<18. 5), normal (18.5 to < 25), overweight (25.0 to < 30), and obese (≥ 30.0) based on cut-points established by the Center for Disease Control (CDC)[16]. Gestational age (GA) was based on the estimated GA noted in EPIC at time of delivery. GA was estimated from the date of the first day of the last menstrual period (if available) together with ultrasounds performed during pregnancy that estimated the gestational age using fetal biometrical measurements. GA was measured in weeks and retained as a continuous variable for adjustment. Race and ethnicity were categorized based on self-reported data from EPIC. Ethnicity was categorized as 'Hispanic' or 'Non-Hispanic' with race categorized as either 'White', 'Black', 'Asian', or 'Other/Mixed'.

Diagnostic criteria for GDM vary by facility depending on the type of testing materials utilized. For this study, GDM status was determined utilizing guidelines based on the American Diabetes Association recommendations through review of laboratory results noted within EPIC records. Glucose tolerance test (GTT) results performed during the second trimester were reviewed and a patient was categorized with GDM if they had a value ≥ 135 mmol/L from a 1 hour GTT, and failed at least one of the steps of a three hour GTT (fasting ≥ 95 mmol/L, 1-hour ≥ 180 mmol/L, 2-hour ≥ 155 mmol/L, or 3-hour ≥ 140 mmol/L)[17].

Statistical Analysis

The underweight and normal BMI categories were combined due to low numbers of subjects in the underweight BMI group noted after the preliminary assessment of the data. Descriptive statistics for continuous variables (e.g., frequencies and proportions) and categorical

variables (e.g., minimum, maximum, median, mean and standard deviation) were presented by fetal sex for each level of GWG. To test the associations between GWG levels and the categorical maternal characteristics, we performed χ^2 tests. To visually assess the linear association between maternal BMI and GWG, scatterplots were created. The least-square means were calculated for each of the GWG levels and plotted with tests for linear trend performed to determine if linear associations existed between GWG and placental weight and umbilical cord length. Poisson regression models were used to estimate the risk ratios of the effect of insufficient or excessive (compared to normal) GWG on each of the categorized placental health outcomes: LGA placental weight, LGA UC length, abnormal UC insertion, and chronic villitis. For these models, both placental weight and umbilical cord length were dichotomized into LGA vs. non-LGA. Linear regression models were constructed to estimate β coefficients, along with standardized β coefficients, for the effect of excessive or insufficient (compared to normal) GWG on gestational age-adjusted placenta weight and UC length. All covariates were included in the full models. P-values < 0.05 were considered significant. Data were analyzed using SAS, version 9.4 for Windows (SAS Institute Inc., Cary, NC).

Results

The descriptive statistics of the 957 patients identified within the Perinatal Biospecimen Repository Cohort that met all inclusion and exclusion criteria for this study are shown in Table 1. The average age of the study cohort was 31.5 years (SD 5.5) with approximately 30% of the cohort greater than 35 years of age and two-thirds of the cohort greater than or equal to 30 years of age. The mean gestational age at delivery was 38.3 weeks (SD 2.9). At time of enrollment, 47.0% of the women were nulliparous. Approximately half (53%) of the cohort had a maternal BMI that was in the overweight or obese categories. The women represented in the study cohort

were more likely to be non-Hispanic (63.6%) than Hispanic (36.4%) and more likely to be white (54.5%) than one of the other assessed races.

Table 1 shows patient characteristics by GWG stratified by fetal sex with 466 female and 491 male fetuses. When examining GWG in pregnancies of a female infant, women with abnormal GWG (i.e., insufficient or excessive) were more likely to be affected by maternal obesity (34.7% and 33.3%, respectively) compared to women with normal GWG (22.4%) In addition, women with excessive GWG were more likely to be overweight (35.1%) than other levels of GWG (normal, 18.8%; insufficient, 12.6%)($p<.0001$). Similar patterns of maternal BMI categories among levels of GWG are shown from pregnancies of male infants.

GWG was shown to be significantly associated with GDM status, for both female and male pregnancies. Women with insufficient GWG and female fetus were more likely to have been diagnosed with GDM (37.8%) during pregnancy compared to other levels of GWG (normal, 26.7%; excessive, 19.5%)($p=0.0020$). Again, similar patterns are reported in both female and male pregnancies.

Within male pregnancies, race was associated with GWG. Asian women were more likely to experience insufficient GWG (18.2%) compared to other levels of GWG (normal, 9.1%; excessive, 6.7%). On the other hand, white and black women were more likely to experience normal and excessive GWG (white: normal, 58.5%; excessive, 57.2%; black: normal, 6.7%; excessive, 5.7%) compared to insufficient GWG (white: 45.5% and black: 3.8%)($p=0.0160$). While the pattern was similar within pregnancies with female infants, the association was not significant ($p=0.1296$).

Figure 3.1 and 3.2 shows a scatterplot of GWG by maternal BMI for pregnancies with female fetuses and pregnancies with male fetuses. Both figures show a negative association between maternal BMI and GWG for both female and male pregnancies. As maternal BMI increases, the amount of weight gained during pregnancy decreases ($p<0.0001$ for all linear associations).

The mean percentiles and 95% CI of gestational age-adjusted placental weight and umbilical cord length by GWG stratified by fetal sex are displayed in Figures 2 and 3. Overall, percentiles of placental weight increased as GWG increased in male pregnancies ($p=0.0073$) with the mean placental weight in the excessive GWG cohort greater compared to both the normal GWG ($p= 0.0030$) and insufficient GWG ($p=0.0274$). In pregnancies with female fetuses, no linear trend was reported between GWG and mean placental weights ($p=0.7059$) as well as no significant difference between levels of GWG. While the association does not appear to be significant for percentiles of UC length, the pattern of the association is similar among both male ($p=0.4179$) and female ($p=0.4400$) pregnancies.

Table 2 shows the estimated effects of GWG on each of the placental outcomes in the crude model and after adjusting for maternal age, parity, GDM status, ethnicity, and race. Based on the crude models, there was significant association between GWG and LGA UC length among pregnancies with male fetuses. This association remained even after adjusting for potential confounders. Women with abnormal GWG had an increased risk of LGA UC length compared to women with normal GWG ($p=0.0033$); specifically, women with excessive GWG had three times the risk of LGA UC length compared to women with normal GWG (aRR: 3.05, 95% CI: 1.45, 6.41) even after adjusting for potential confounders.

In addition, we found a significant positive association between GWG and gestational age-adjusted UC length among pregnancies with male fetuses (($\beta = 0.2011$, $p = 0.0492$) after adjustment for confounding (Table 3). For every one pound increase in gestational weight, the percentile UC length for male fetuses increased on average by 0.2018. No such association was noted within pregnancies with female fetuses. GWG was not found to be associated with an altered risk of LGA placental weight in the cohort overall (Table 2), but we did find a significant positive association between GWG and gestational age-adjusted placental weight among male pregnancies ($\beta = 0.3008$, $p = 0.0022$) after adjustment for confounding (Table 3). For every one pound increase in gestational weight, the percentile of placental weight increased by 0.3008 for male fetuses. Again, no association between GWG and placental weight was noted among female pregnancies.

Discussion

This study assessed the effect of GWG on various measurements of placental health and function including placental weight, length of umbilical cord, umbilical cord insertion and chronic villitis in a cohort of women treated within an academic medical center. In agreement with our hypotheses, GWG was associated with an increased risk of placental weight and UC length and those risks varied by fetal sex. We found that increased GWG was significantly associated with increased placental weight and increased UC length and that the magnitude of the risk was greater in pregnancies with male fetuses.

The magnitudes of the observed effects in the pregnancies with male fetuses are clinically relevant. GWG was positively associated with increased risk of large placental weight and long UC length even adjusting for gestational age, suggesting that increases in gestational weight over

the pregnancy increased the risk of developing large-for-gestational age placentae and umbilical cords in pregnancies with male fetuses. Additional analyses of pregnancies with a male fetus indicated that women with excessive GWG had three times the risk of developing a large-for-gestational age umbilical cord during pregnancy as compared to women with normal GWG.

While current research regarding the effect of GWG on the length of the umbilical cord is limited and even more scarce assessing the effects by fetal sex, excessive GWG has been linked to alterations in gene expression associated with inflammation, insulin resistance and epigenetic programming through DNA methylation within the placenta, umbilical cord, and cord blood [11-13]. Our study suggests that women experiencing excessive GWG with a male fetus have three times the risk of developing a longer than normal umbilical cord, which has been shown to be associated with delivery complications as well as cord prolapse, torsion, and true knot entanglement[18].

While the linear association between GWG and placental weight was significant, that association did not maintain significance comparing excessive GWG to normal GWG in regards to an LGA placental weight. Even so, the trend was similar for males whereas it was not in pregnancies with female fetuses.

No significant findings were reported for pregnancies with female fetuses. The variability in the effect of maternal characteristics on placental health based on fetal sex is poorly understood. Leon-Garcia, et. al. (2015) found varying associations between maternal obesity and inflammatory markers in placental tissue by fetal sex. Their study reported that women with maternal obesity and a female fetus had higher occurrences of chronic villitis compared to pregnancies with a male fetus[19]. In regards to GWG, our results did not show an increase in CV

in pregnancies with excessive GWG with either a female or male fetus. The difference in the association between maternal obesity and excessive GWG with CV could be influenced by the timing of the exposures. Maternal BMI is measured at the beginning of the pregnancy, whereas GWG is measured at the end of the pregnancy and fluctuates throughout gestation. While GWG is dependent on pregravid BMI, the potential effect GWG has on the risk of CV might be difficult to elucidate. It is hypothesized that the presence of chronic villitis within the placentas of female fetuses could dampen the effects maternal obesity and excessive weight gain have on placental measurements. Further analyses are needed to determine if an inflammatory state can have an impact on placental size or umbilical cord length.

There are several limitations to our study. Our cohort represents a group of women who were more likely to receive prenatal care and deliver at a regional academic care center, which might have limited generalizability. Women without a maternal weight measurement during the first 14 weeks of gestation or lacking a recorded maternal weight near delivery were excluded from this study. These women might represent patients with minimal prenatal care, which has been linked to poor birth and maternal outcomes at delivery.

Nevertheless, this study also has numerous strengths. Our sample size was large, allowing for stratification by variables of interest, including BMI, ethnicity, race, and GDM, as well as fetal sex. The ability to abstract information from both the Perinatal Repository and medical records in EPIC provided the opportunity to create a robust data set that allowed for the incorporation of various potential confounders, both demographic and clinical

In conclusion, our findings show that amount of weight gained during gestation is associated with the health of the placenta, especially women experiencing excessive weight gain

during pregnancy. Women who become pregnant may benefit from monitoring and maintaining their weight gain during gestation complying with the standards set by IOM in order to reduce the risk of poor placental health outcomes. Our findings show that even small decreases in weight gain may have a positive impact on umbilical cord lengths, especially in pregnancies with male fetuses. Furthermore, fetal sex can be used to help identify women at high risk for abnormal placentas and umbilical cords in the presence of excessive weight gain, which could provide the opportunity for early intervention.

Acknowledgments

Chapter 3, in full, is currently being prepared for submission for publication of the material. Rush, Toni M; Laurent, Louise C; Quintana, Penelope J; Jain, Sonia; Thompson, Caroline A; Parast, Mana; Chambers, Christina D. Toni Rush was the primary investigator and author of this material.

Table 3-1 Patient Characteristics by Placental Outcomes for Pregnancies with Female Fetuses, 2010-2015 (n=469)

		FEMALE						MALE					
		Unadjusted RR			Adjusted RR			Unadjusted RR			Adjusted RR		
Outcome*	**GWG**	**RR**	**95% CI**	**p-value**	**RR**	**95% CI**	**p-value**	**aRR**	**95% CI**	**p-value**	**aRR**	**95% CI**	**p-value**
LGA Placental Weight				0.9107			0.9932			0.0786			0.0892
	Insufficient	1.13	(0.50,2.53)		0.98	(0.42,2.26)		0.70	(0.28, 1.71)		0.72	(0.29, 1.79)	
	Normal	--	--		--	--		--	--		--	--	
	Excessive	0.94	(0.44, 2.05)		0.95	(0.43, 2.11)		1.67	(0.84, 3.31)		1.70	(0.85, 3.39)	
LGA UC Length				0.4498			0.1954			**0.0090**			**0.0033**
	Insufficient	0.82	(0.31, 2.17)		0.66	(0.24, 1.80)		1.15	(0.47, 2.79)		1.13	(0.46, 2.81)	
	Normal	--	--		--	--		--	--		--	--	
	Excessive	1.42	(0.64, 3.15)		1.57	(0.69, 3.58)		**2.66**	**(1.29, 5.48)**		**3.05**	**(1.45, 6.41)**	
Abnormal UC Insertion				0.2242			0.1101			0.3287			0.1577
	Insufficient	1.62	(0.82, 3.19)		1.81	(0.90, 3.64)		1.65	(0.83, 3.29)		2.01	(0.99, 4.09)	
	Normal	--	--		--	--		--	--		--	--	
	Excessive	0.94	(0.47, 1.88)		0.90	(0.45, 1.81)		1.47	(0.77, 2.79)		1.43	(0.74, 2.75)	
Chronic Villitis				0.2673			0.3198			0.6902			0.6470
	Insufficient	1.44	(0.78, 2.64)		1.38	(0.74, 2.56)		1.12	(0.59, 2.09)		1.09	(0.58, 2.07)	
	Normal	--	--		--	--		--	--		--	--	
	Excessive	1.56	(0.90, 2.73)		1.53	(0.87, 2.69)		1.28	(0.73, 2.23)		1.30	(0.74, 2.29)	

Model adjusted for maternal age, parity, ethnicity, race, GDM status. Abbreviations: RR – relative risk, aRR – adjusted relative risk, CI – confidence interval, GWG – Gestational Weight Gain, LGA – large for gestational age, UC – umbilical cord; * Reference Non-LGA Placental Weight, Non-LGA UC Length, Normal UC Insertion, No Villitis

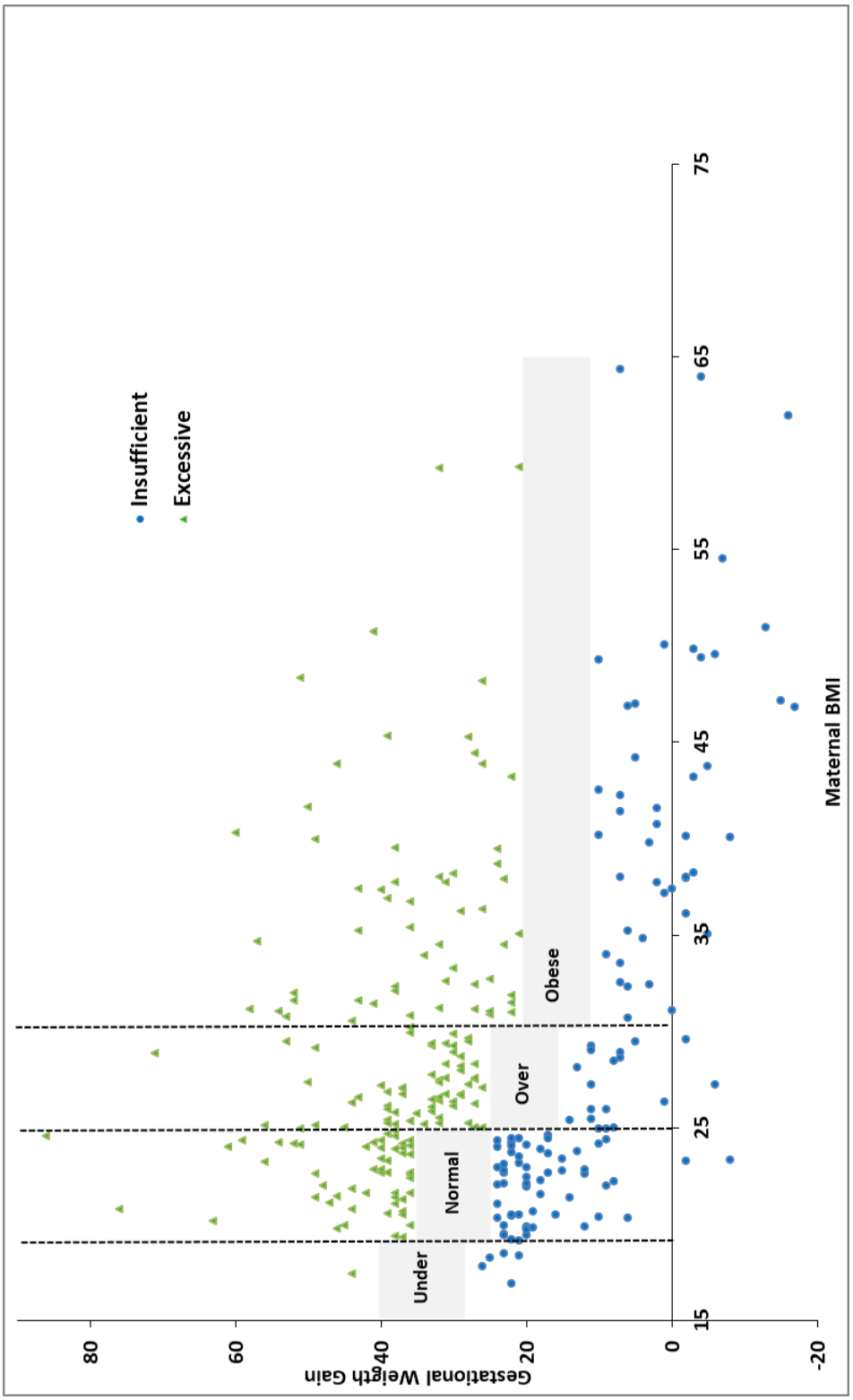

Figure 3-1 Gestational weight gain by maternal BMI for FEMALE fetal sex ($p<0.0001$ for all linear associations). Shaded areas are recommended ranges of weight gain for each BMI category. NOTE: x-axis and y-axis range does not begin at zero

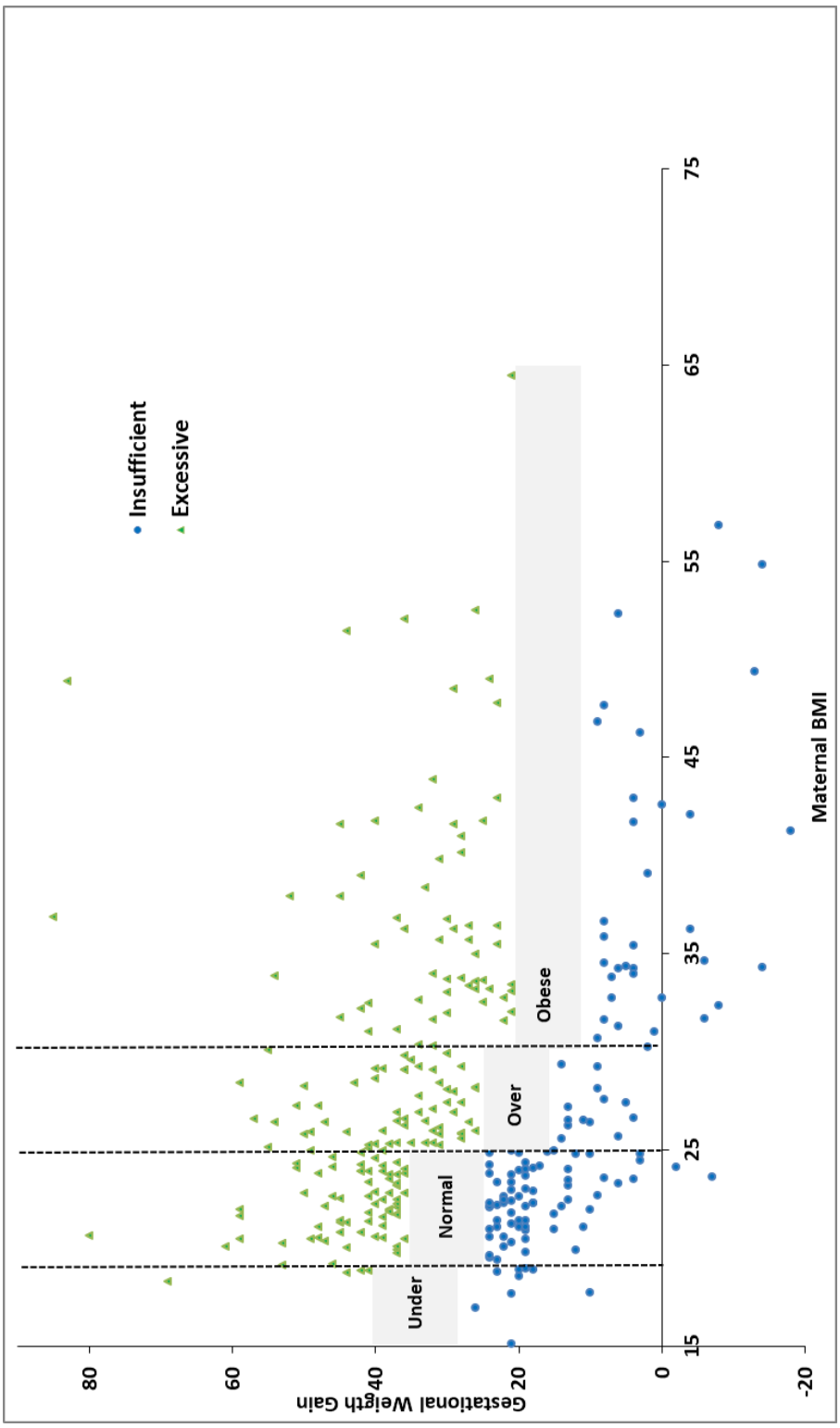

Figure 3-2 Gestational weight gain by maternal BMI for MALE fetal sex ($p<0.0001$ for all linear associations). Shaded areas are recommended ranges of weight gain for each BMI category. NOTE: x-axis and y-axis range does not begin at zero.

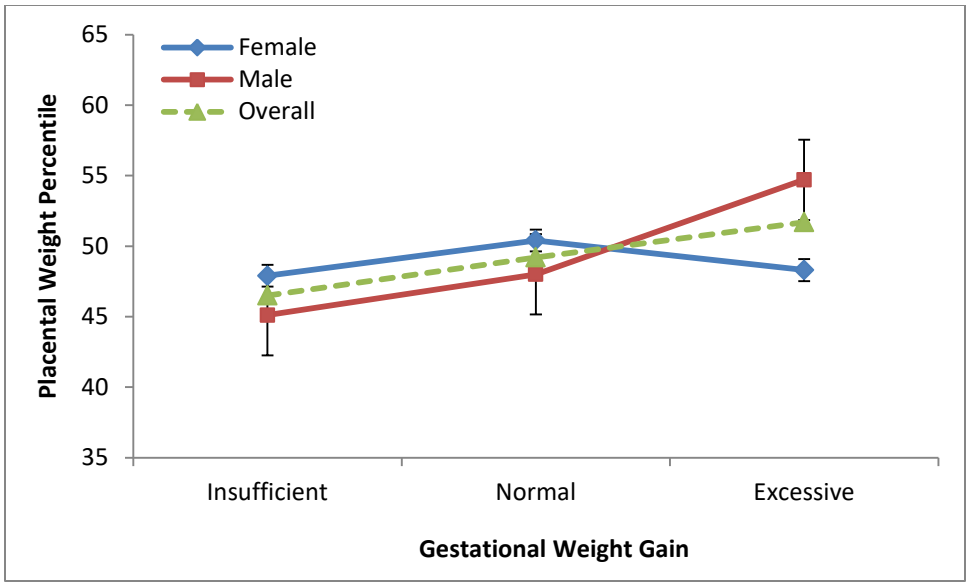

Figure 3-2 Gestational age-adjusted percentile of placental weight by gestational weight gain stratified by fetal sex(Female, p=0.7059; Male, p=0.0073). Note: p-values represent linear test for trend; y-axis range does not begin at zero.

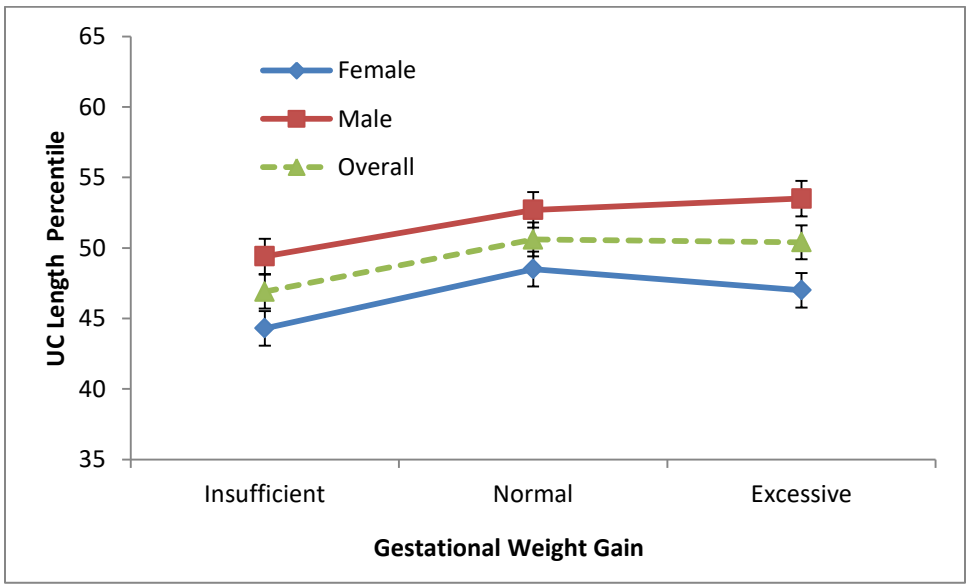

Figure 3-3 Gestational age-adjusted percentile of umbilical cord (UC) length by gestational weight gain stratified by fetal sex (Female, p=0.4400; Male, p=0.4179). Note: p-values represent linear test for trend; y-axis range does not begin at zero.

Table 3-2 Risk Ratios of Gestational Weight Gain effect on Placental Health Outcomes by Fetal Sex, 2010-2015 (n=957)

		FEMALE						MALE					
		Unadjusted RR			Adjusted RR			Unadjusted RR			Adjusted RR		
Outcome*	**GWG**	**RR**	**95% CI**	**p-value**	**RR**	**95% CI**	**p-value**	**aRR**	**95% CI**	**p-value**	**aRR**	**95% CI**	**p-value**
LGA Placental Weight				0.9107			0.9932			0.0786			0.0892
	Insufficient	1.13	(0.50,2.53)		0.98	(0.42,2.26)		0.70	(0.28, 1.71)		0.72	(0.29, 1.79)	
	Normal	--	--		--	--		--	--		--	--	
	Excessive	0.94	(0.44, 2.05)		0.95	(0.43, 2.11)		1.67	(0.84, 3.31)		1.70	(0.85, 3.39)	
LGA UC Length				0.4498			0.1954			**0.0090**			**0.0033**
	Insufficient	0.82	(0.31, 2.17)		0.66	(0.24, 1.80)		1.15	(0.47, 2.79)		1.13	(0.46, 2.81)	
	Normal	--	--		--	--		--	--		--	--	
	Excessive	1.42	(0.64, 3.15)		1.57	(0.69, 3.58)		**2.66**	**(1.29, 5.48)**		**3.05**	**(1.45, 6.41)**	
Abnormal UC Insertion				0.2242			0.1101			0.3287			0.1577
	Insufficient	1.62	(0.82, 3.19)		1.81	(0.90, 3.64)		1.65	(0.83, 3.29)		2.01	(0.99, 4.09)	
	Normal	--	--		--	--		--	--		--	--	
	Excessive	0.94	(0.47, 1.88)		0.90	(0.45, 1.81)		1.47	(0.77, 2.79)		1.43	(0.74, 2.75)	
Chronic Villitis				0.2673			0.3198			0.6902			0.6470
	Insufficient	1.44	(0.78, 2.64)		1.38	(0.74, 2.56)		1.12	(0.59, 2.09)		1.09	(0.58, 2.07)	
	Normal	--	--		--	--		--	--		--	--	
	Excessive	1.56	(0.90, 2.73)		1.53	(0.87, 2.69)		1.28	(0.73, 2.23)		1.30	(0.74, 2.29)	

Model adjusted for maternal age, parity, ethnicity, race, GDM status. Abbreviations: RR – relative risk, aRR – adjusted relative risk, CI – confidence interval, GWG – Gestational Weight Gain, LGA – large for gestational age, UC – umbilical cord; * Reference Non-LGA Placental Weight, Non-LGA UC Length, Normal UC Insertion, No Villitis

Table 3-3 Association of gestational weight gain with gestational age-adjusted placental weight and umbilical cord length stratified by fetal sex; (n=957)

Model[a]	Placental Weight			Umbilical Cord Length		
	β	Standardized β	p-value	β	Standardized β	p-value
Crude Model						
Female	0.0932	0.0480	0.3319	0.0534	0.0279	0.5772
Male	0.3090	0.1536	**0.0010**	0.2018	0.1001	**0.0365**
Full Model						
Female	0.1098	0.0565	0.2799	0.1101	0.0574	0.2791
Male	0.3008	0.1495	**0.0022**	0.2011	0.0997	**0.0492**

[a] Crude model: adjusted for maternal BMI. Full model: adjusted for maternal BMI, maternal age, parity, ethnicity, race, GDM status.

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Chapter 4

Fetal sex modifies the effect of maternal BMI on multiple indicators of placental health

Background: The pregravid BMI of a woman can impact various characteristics of the placenta that can be used to indicate the health of the placenta during gestation. The modification by fetal sex of these effects is poorly defined.

Objective(s): This study aimed to determine if the effects maternal BMI have upon indicators of placental health are modified by the sex of the fetus *in utero*.

Study Design: This retrospective cohort study used data from the University of California San Diego Perinatal Biospecimen Repository Cohort collected from patients between September 1, 2010 to August 31, 2015. Eligible patients were at least 18 years old with a measured maternal height and weight in the first 14 weeks of gestation. Those with pre-existing Type I or Type II diabetes mellitus were excluded from the study. Outcomes of interest included placental weight, umbilical cord length, umbilical cord insertion, and chronic villitis. All analyses were stratified by fetal sex. Poisson regression models were used to estimate the risk ratios of maternal BMI and placental health measurements by fetal sex.

Results: Within the Perinatal Biospecimen Repository Cohort, 965 patients were identified to meet the study criteria. Of the overall study cohort, there were 469 pregnancies with female fetuses and 496 pregnancies with male fetuses with similar characteristics for both cohorts. The average age of the cohort was 31 years old (SD 5) with approximately half of either of the two cohorts had maternal BMI that were categorized as overweight or obese. In pregnancies with

male fetuses, patients with pregravid obesity had approximately two and a half times the risk (aRR: 2.52, 95% CI: 1.33, 4.77; p = 0.0044) of having an LGA placenta and three times the risk of LGA umbilical cord length (aRR: 3.01, 95% CI: 1.56, 5.80; p = 0.0010) compared to women without pregravid obesity. No significant difference was reported for LGA placentas and LGA umbilical cord lengths in pregnancies with female fetuses. Rather in pregnancies with female fetuses, patients with pregravid obesity had an 80% increase in risk (aRR: 1.79, 95% CI: 1.05, 3.04; p = 0.0313) of CV compared to women without pregravid BMI. This association was not shown in pregnancies with male fetuses. Abnormal cord insertion was not significantly different for either fetal sex.

Conclusion(s): Maternal BMI affects various indicators of placental health that are measured at birth. Interestingly, these effects are modified by the sex of the fetus. In regards to pregnancies affected by pregravid BMI, pregnancies with male fetuses are at higher risk of increased placental and umbilical cord size, while pregnancies with female fetuses are at higher risk of CV, a marker for inflammation in the placental tissue.

Fetal sex modifies the effect of maternal BMI on multiple indicators of placental health

Background

The prevalence of obesity is approximately 40% within the female population in the United States with slight variation among women of child-bearing age, with 37% of women less than 40 years of age having obesity compared to women greater than 40 years of age with a prevalence of 45%.[1] Obesity is associated with a chronic state of inflammation[2] as well as

increased risks of various medical conditions such as hypertension, coronary heart disease, Type 2 diabetes mellitus and stroke.[3,4] Due to the initiation of placental development occurring at implantation which precedes embryonic development, the mother's health at conception is vital to the development of the placenta and in turn the overall pregnancy.

While pregravid obesity has been shown to increase the risk of negative birth outcomes (e.g., large for gestational age, LGA)[5,6] in infants, the effects of maternal obesity on placental development and function are less understood. Nevertheless, there is growing evidence that fetal sex modifies the effects that maternal exposures have on birth outcomes such as LGA or small for gestational age (SGA). In uncomplicated singleton pregnancies, research suggests that intrauterine growth patterns vary by fetal sex.[7] Female fetuses grow at a slower rate compared to male fetuses with growth patterns also varying between the two sexes.[7] In regards to less than optimal maternal nutrition, a recent study reported that the effects of maternal macronutrient intake was more pronounced in male fetuses compared to female fetuses.[8] The weight of the infant at birth is dependent on the nutritional exchange between the mother and fetus which is facilitated by the placenta and umbilical cord. Recent studies have reported that maternal obesity is associated with inflammation in placental tissue examined after birth.[2,9] One study's findings found that maternal obesity is associated with chronic villitis and that this lesion was more common in the placentas of female offspring.[9] Other research supporting the variability in placental outcomes between the sex of the offspring is growing..

We hypothesize that the effects on placental development stimulated by maternal obesity which influences the intrauterine environment are modified by the sex of the fetus during pregnancy. In addition, we hypothesize that the effect obesity has on the development of the placenta is attenuated in the presence of chronic villitis. In this study, we investigated the

modification of the effect by fetal sex of pregravid BMI on placental function in a study cohort of pregnant women with linked demographic and clinical data, as well as placental pathology data, allowing adjustment for clinical characteristics.

Methods

This retrospective cohort study utilized data from the University of California San Diego (UCSD) Perinatal Biospecimen Repository Cohort[10] over a 5-year study period, from September 1, 2010 to August 31, 2015. In the Perinatal Biospecimen Repository Cohort, both low- and high- risk pregnant patients were consented and enrolled to obtain clinical data and multiple biospecimens, including placental tissue. Women who were at least 18 years old at time of delivery and had a measured maternal height and weight in the first 14 weeks of gestation were included in the study. Women with pre-existing Type 1 diabetes mellitus (T1DM) or T2DM, were excluded from the study. Maternal demographic, anthropometric, and obstetric data, as well as neonatal outcome data, were abstracted from the electronic health record (EHR) data source housed in EPIC (Epic Systems Corporation, Verona, WI).

This study was approved by the Human Research Protections Program of the University of California San Diego; all participants provided written informed consent prior to participation in the Perinatal Biospecimen Repository Cohort.

Outcome Assessment

The four pathologic outcomes assessed during the study were: placental weight; umbilical cord length; umbilical cord insertion; and a histological finding of chronic villitis (CV).[11] Each of these outcomes was assessed after delivery. Placental gross and microscopic

examination and preparation of slides for histology were supervised by the same Board-Certified Anatomic Pathologist with fellowship training in Perinatal Pathology (M. Parast).

For placental weight and umbilical cord length, gestational age-adjusted percentiles based on published nomograms were calculated, and then the percentiles were used to categorize the placental weights and umbilical cord lengths into three levels: small for gestational age (SGA), appropriate for gestational age (AGA), and large for gestational age (LGA).

Umbilical cord insertion was dichotomized into 'Normal' (for central cord insertions) and 'Abnormal' (for marginal, velamentous, or furcate cord insertions) based on the location of the insertion point of the umbilical cord to the placenta. Finally, a diagnosis of CV was categorized as either present or absent by the reviewing pathologist, regardless of the grade of the lesion.

Covariate Assessment

All relevant covariates were determined based on the use of a directed acyclic graph (DAG) as an aid in determining confounders and mediators.[12] The primary exposure of interest for this study was maternal BMI. Maternal BMI was calculated using a maternal weight and height recorded at less than 14 weeks gestation, with the earliest available measurement used for analysis. Maternal BMI was categorized based into four groups: underweight (<18.5), normal (18.5 to < 25), overweight (25.0 to < 30), and obese ($\geq$ 30.0).[13] Possible confounders included in our analysis were maternal age, parity, gestational age (GA) at delivery, GDM status, and mother's self-reported race and ethnicity. Maternal age at the time of conception was calculated using the mother's birthdate and date of conception based on estimated gestational age from the earliest recorded date for prenatal care. Parity, i.e., number of deliveries after 20 weeks' gestation, was coded based on self-reported data and remained an ordinal variable in analyses.

Using EPIC, GA at delivery was estimated from the date of the first day of the last menstrual period (if available) together with fetal biometrical measurements taken during ultrasounds. GA was measured in weeks and maintained as a continuous variable during analyses. Maternal ethnicity was categorized as 'Hispanic' or 'Non-Hispanic', and race categorized as either 'White', 'Black', 'Asian', or 'Other' both of which were based on self-reported data from EPIC. Fetal sex was a dichotomous variable noted as either 'Male' or 'Female'.

For this study, GDM status was determined through review of laboratory results noted within EPIC records and applying guidelines based on American Diabetes Association recommendations. Glucose tolerance test (GTT) results performed during the second trimester were reviewed and a woman was categorized as having GDM if she had a value ≥135 mmol/L from a 1 hour GTT, and failed at least two of the steps of a three hour GTT (fasting ≥95 mmol/L, 1-hour ≥180 mmol/L, 2-hour ≥155 mmol/L, or 3-hour ≥140 mmol/L).[14]

Statistical Analysis

For this study, all analyses were stratified by fetal sex. Underweight and normal BMI categories were combined due to low numbers of subjects in the underweight BMI level. Descriptive statistics for continuous and categorical variables were presented by each of the outcomes. We performed χ^2 tests to assess the association between maternal BMI and maternal characteristics. The least-square means were calculated for placental weight and umbilical cord length among each of the BMI categories and plotted with tests for linear trend performed. To assess the linear association between maternal BMI and gestational-age adjusted placental weight and umbilical cord length, generalized linear models were constructed to estimate β coefficients. Poisson regression models were used to estimate the risk ratios of the effect

between pregravid obesity and each of the placental health outcomes. For these models, both placental weight and umbilical cord length were dichotomized into LGA vs. non-LGA. All covariates were included in the full models. Maternal BMI was also assessed as continuous in Poisson regression models to estimate the effect size of the association between maternal BMI and abnormal UC insertion and CV. Data were analyzed using SAS, version 9.4 for Windows (SAS Institute Inc., Cary, NC).

Results

Table 1 and Table 2 show the descriptive statistics for the patients who were identified from the Perinatal Biospecimen Repository Cohort that met all study criteria stratified by fetal sex. Of the overall cohort, there were 469 pregnancies with female fetuses and 496 pregnancies with male fetuses. The characteristics were similar for pregnancies of both female and male fetuses. The average age of the female fetus cohort was 31.4 years old (SD 5.5) and the male fetus cohort was 31.5 years old (SD 5.4) with 65% of both cohorts greater than 30 years of age. The mean gestational age at delivery within both cohorts was 38 weeks (SD 3). Approximately half (51%) of the cohorts had a BMI that was in the overweight or obese categories. Ethnicity and race did not vary by the pregnancy cohorts with women represented in the both cohorts were more likely to be non-Hispanic and white than Hispanic or other races.

In pregnancies with female fetuses and affected by maternal obesity, CV was more likely to be present (42.7% with, and 26.8% without) ($p = 0.0012$)(Table 1). This trend was similar but not significant in pregnancies with a male fetus ($p = 0.4014$) (Table 2). In pregnancies with male fetuses and affected by maternal obesity, placentas were more likely to be LGA vs. non-LGA (LGA, 48.0 %; AGA, 29.8%; SGA, 11.5%)($p = 0.0010$)(Table 2). In pregnancies with female

fetuses, maternal BMI was not significantly associated with placental weight (p=0.1992). Similar trends among the two study cohorts were noted in the association between maternal BMI and UC length. LGA umbilical cords were more likely in pregnancies affected by maternal obesity compared to non-LGA umbilical cords (LGA, 44.2 %; AGA, 29.1%; SGA, 18.4%) in pregnancies with a male fetus(p = 0.0348)(Table 2). In pregnancies with a female fetus, a borderline significant association was found between maternal BMI and UC length (LGA, 38.2%; AGA, 30.6%; SGA, 18.4%; p = 0.0593)(Table 1).

Figure 1 displays the mean percentiles and 95% CI of gestational age-adjusted placental weight by maternal BMI stratified by fetal sex. Percentiles of placental weight in pregnancies with female fetuses (p=0.0008, linear trend) and male fetuses (p<0.0001, linear trend) increased as maternal BMI increased. Figure 2 displays the mean percentiles and 95% CI of gestational age-adjusted UC length by maternal BMI stratified by fetal sex. No linear trend was reported for either cohort.

There were no significant association between maternal obesity and LGA placental weight and LGA UC length after adjustment for potential confounders in pregnancies with female fetuses (Table 3). In pregnancies with a female fetus, the risk of CV in women with obesity was increased by 79% (aRR: 1.79, 95% CI: 1.05, 3.04; p=0.0313) compared to women with underweight/ normal or overweight maternal BMI.

Based on the results shown in Table 3, there were significant associations between maternal obesity and LGA placental weight and LGA UC length both before and after adjustment for potential confounders in pregnancies with male fetuses. Women with maternal obesity had over twice the risk (aRR: 2.52, 95% CI: 1.33, 4.77; p = 0.0044) of having an LGA

placental weight compared to women with lower reported maternal BMI (i.e., underweight/normal, overweight). In pregnancies with a male fetus, the relative risk for LGA umbilical cord length was significantly increased in women with obesity, with a tripling of the risk (aRR: 3.01, 95% CI: 1.56, 5.80; p=0.0010) compared to women with lower maternal BMI after model adjustment. The risk of CV was not increased in pregnancies affected by maternal obesity with a male fetus.

Of the cohort of pregnancies with a female fetus, 140 (29.9%) were affected by maternal obesity with 38 (27.1%) of those being diagnosed with CV. More SGA placentas were diagnosed with CV (41.7%) than LGA (33.3%). A similar trend was seen in the cohort of pregnancies with a male fetus affected by maternal obesity (147, 29.6%) and CV (30, 20.4%). More SGA placentas were diagnosed with CV (33.3%) than LGA (13.0%)(Table 4).

Discussion

This study evaluated the effects pregravid BMI has on placental health stratified by fetal sex in women receiving care at an academic care center. The findings of the study support our hypothesis that increased maternal BMI increases the risk of abnormal placental health and that the magnitude of the risks vary depending on the sex of the fetus. We found that in pregnancies with a female fetus, the risk of CV is increased in women with obesity, while the risks of abnormal measurements in other indicators of placental health were not increased. This varied in pregnancies with male fetuses, where the risk of LGA placentas and LGA umbilical cords were increased in women with maternal obesity, but no increased risk of CV was reported.

The findings from this study are clinically relevant. Maternal BMI was significantly associated with various abnormal placental findings and those associations were modified by the

sex of the fetus even after adjusting for maternal age, parity, ethnicity, and race. Further analysis of pregnancies with a male fetus indicated that women with pregravid obesity had two and a half times the risk of having an LGA placenta compared to women without obesity even after multivariate adjustment for confounding. No such association was found among pregnancies with female fetuses. A previous study by Leon-Garcia et al (2015) reported similar associations between maternal obesity and large placental weight, noting that pregravid obesity more than doubled the risk of large placentas.[9] Even though this earlier study used subjects from the same Perinatal Repository with similar results, they did not stratify by fetal sex nor adjust for confounding by demographic characteristics known to be associated with maternal BMI and birth outcomes, such as maternal race and ethnicity. This might explain why our effect size was similar with the dampening of their results due to lack of stratification and the lack of confounding which results in a slightly inflated effect size.

In addition, our study indicated that women with pregravid obesity pregnant with male fetuses have three times the risk of LGA umbilical cord compared to women with lower pregravid BMI. Again, no association between maternal BMI and umbilical cord length was found in pregnancies with female fetuses. While current literature is scarce regarding the effect of maternal BMI on the length of the umbilical cord at birth with modification by fetal sex even more remote, alteration in gene expression and epigenetic programming through DNA methylation within the placenta and cord blood have been linked to maternal obesity.[16,17] Even though these variations of genetic expression and global methylation are not necessarily correlated with the length of the umbilical cord, these results allow the generation of a hypothesis that maternal obesity influences genetic expression and DNA methylation that do correlate with the developmental pathways of the umbilical cord. The increased risk reported in our study of a

longer than normal umbilical cord has been shown to impact delivery complications such as cord prolapse, torsion, and true knot entanglement.[18]

Subsequent analyses showed that women with pregravid obesity and pregnant with a female fetus had an 80% increase in the risk of CV, while no significant risk of CV was reported in women with obesity carrying a male fetus. These findings are supported by our own previous research, showing that maternal obesity was associated with an increase in inflamed placental tissues, and that the magnitude of the effect was higher in pregnancies with a female fetus.[9] While the presence of CV is an indicator of inflammation in the placental tissue, its clinical relevance is being elucidated. The grade of these lesions varies along with the impact on placental function.[19] Low-grade lesions are not as clinically severe, compared to higher grade lesions that have been linked to fetal growth restriction.[20]

Due to the link to fetal growth restriction, it has been hypothesized that CV could attenuate the effects maternal obesity has on placental outcomes, especially larger than normal placentas and/or umbilical cords. Our results that show an increase in risk of CV in female fetuses, but not increased risk in placental weight or umbilical cord length. The opposite is found in male fetuses. These data suggest that this hypothesis deserves further assessment. While the current study can neither support nor reject this hypothesis, the results do provide insight that further research is warranted to determine if the inflammatory state manifested as CV dampens the negative effects obesity has on placental health, and if that increased risk of CV outweighs the increased risk of abnormal placentas and umbilical cords.

There are limitations to our study. Our cohort represents a group of women who were more likely to receive prenatal care and deliver at a regional academic care center, which might

have limited generalizability. Women without a maternal weight measurement during the first 14 weeks of gestation were excluded from this study. These women might represent patients with minimal prenatal care, which has been linked to poor birth and maternal outcomes at delivery.

Nevertheless, this study also has numerous strengths. Our sample size was large, allowing for stratification by fetal sex and assessment of multiple variables. The ability to abstract information from both the Perinatal Repository and medical records in EPIC provided the opportunity to create a robust data set that allowed for the incorporation of various potential confounders, both demographic and clinical. In addition, all placentas were evaluated in a blinded manner with respect to maternal BMI, by a single pathologist specializing in placental assessment, providing consistent results and avoiding interobserver variability.

Our findings show that maternal BMI is associated with the health of the placenta and that the effects are modified by fetal sex. Women in their child-bearing years who are considering becoming pregnant may benefit from decreasing their weight in an attempt to reduce the risk of poor placental health outcomes. Women at high risk for abnormal placentas and umbilical cords can be identified based on a woman's BMI prior to conception. Furthermore, our findings suggest that the sex of the fetus within pregnancies affected by pregravid obesity can be a marker for specialized surveillance of the placenta and umbilical cord during pregnancy. By identifying at-risk women, the opportunity to reduce poor birth outcomes with early intervention becomes possible.

Acknowledgments

Chapter 4, in full, is currently being prepared for submission for publication of the material. Rush, Toni M; Laurent, Louise C; Quintana, Penelope J; Jain, Sonia; Thompson, Caroline A; Parast, Mana; Chambers, Christina D. Toni Rush was the primary investigator and author of this material.

Table 4-1 Patient Characteristics by Placental Outcomes for Pregnancies with Female Fetuses, 2010-2015 (n=469)

	Overall	Placental Weight				Cord Length				Chronic Villitis		
Characteristics	**(n=469) N (%)**	**SGA (n=49) N (%)**	**AGA (n=379) N (%)**	**LGA (n=41) N(%)**	**p-value**	**SGA (n=49) N(%)**	**AGA (n=386) N(%)**	**LGA (n=34) N(%)**	**p-value**	**No (n=380) N(%)**	**Yes (n=89) N(%)**	**p-value**
Maternal BMI	28.0 mean, 8.2 SD				0.1992				0.0593			**0.0012**
Underweight/Normal	220 (46.9)	27(55.1)	176(46.4)	17(41.5)		30(61.2)	180(46.6)	10(29.4)		193(50.8)	27(30.3)	
Overweight	109 (23.2)	10(20.4)	93(24.5)	6(14.6)		10(20.4)	88(22.8)	11(32.4)		85(22.4)	24(27.0)	
Obese	140 (29.9)	12(24.5)	110(29.0)	18(43.9)		9(18.4)	118(30.6)	13(38.2)		102(26.8)	38(42.7)	
Maternal age (years)	31.5 mean, 5.6 SD				0.3219				0.7391			0.**0656**
18-24	55 (11.7)	5(10.2)	43(11.3)	7(17.1)		6(12.2)	47(12.2)	2(5.9)		41(10.8)	14(15.7)	
25-29	106 (22.6)	13(26.5)	79(20.8)	14(34.2)		9(18.4)	87(22.5)	10(29.4)		90(23.7)	16(18.0)	
30-34	165 (35.1)	17(34.7)	139(36.7)	9(22.0)		16(32.7)	139(36.0)	10(29.4)		126(33.2)	39(43.8)	
35+	143 (30.4)	14(28.6)	118(31.1)	11(26.8)		18(36.7)	113(29.3)	12(35.3)		123(32.4)	20(22.5)	
Parity	1.8 mean, 0.9 SD				**0.0485**				0.**0409**			0.3938
0	219 (46.6)	26(53.1)	180(47.5)	13(31.7)		30(61.2)	180(46.6)	9(26.5)		183(48.2)	36(40.5)	
1	148 (31.6)	16(32.7)	120(31.7)	12(29.7)		11(22.5)	122(31.6)	15(44.1)		117(30.8)	31(34.8)	
2+	102 (21.7)	7(14.3)	79(20.8)	16(39.0)		8(16.3)	84(21.8)	10(29.4)		80(21.1)	22(24.7)	
GDM					0.6138				0.1364			0.8946
No	343 (73.1)	38(77.6)	277(73.1)	28(68.3)		40(81.6)	282(73.1)	21(61.8)		277(72.9)	66(74.2)	
Yes	126 (26.9)	11(22.5)	102(26.9)	13(31.7)		9(18.4)	104(26.9)	13(38.2)		103(27.1)	23(25.8)	
Ethnicity					**0.0498**				0.2277			**0.0199**
Non-Hispanic	299 (63.7)	36(73.5)	243(64.1)	20(48.8)		32(65.3)	250(64.8)	17(50.0)		252(66.3)	47(52.8)	
Hispanic	170 (36.2)	13(26.5)	136(35.9)	21(51.2)		17(34.7)	136(35.2)	17(50.0)		128(33.7)	42(47.2)	
Race					0.5463				0.6311			0.571
White	255 (54.3)	29(59.2)	201(53.0)	25(60.1)		25(51.0)	214(55.4)	16(47.1)		209(55.0)	46(51.7)	
Black	29 (6.1)	4(8.2)	24(6.3)	1(2.4)		3(6.1)	24(6.2)	2(5.9)		24(6.3)	5(5.6)	
Asian	56 (11.9)	6(12.2)	46(12.1	4(9.8)		6(12.2)	48(12.4)	2(5.9)		47(12.4)	9(10.1)	
Other/Mixed	129 (27.5)	10(20.4)	107(28.2)	12(29.3)		15(30.6)	100(25.9)	14(41.2		100(26.3)	29(32.6)	

Table 4-2 Patient Characteristics by Placental Outcomes for Pregnancies with Male Fetuses, 2010-2015

Characteristics	Overall (n=496) N (%)	Placental Weight SGA (n=52) N (%)	AGA (n=396) N (%)	LGA (n=48) N(%)	p-value	Cord Length SGA (n=49) N(%)	AGA (n=395) N(%)	LGA (n=52) N(%)	p-value	Chronic Villitis No (n=412) N(%)	Yes (n=84) N(%)	p-value
Maternal BMI	28.0 mean, 8.2 SD				**0.0010**				**0.0348**			0.4014
Underweight/Normal	243 (49.0)	36(69.2)	189(47.7)	18(37.5)		25(51.0)	195(49.4)	23(44.2)		206(50.0)	37(44.1)	
Overweight	106 (21.4)	10(19.2)	89(22.5)	7(14.6)		15(30.6)	85(21.5)	6(11.5)		89(21.6)	17(20.2)	
Obese	147 (29.6)	6(11.5)	118(29.8)	23(48.0)		9(18.4)	115(29.1)	23(44.2)		117(28.4)	30(35.7)	
Maternal age (years)	31.4 mean, 5.5 SD				0.8394				0.2903			0.1452
18-24	61 (12.3)	4(7.7)	50(12.6)	7(14.6)		4(8.2)	55(13.9)	2(3.9)		55(13.4)	6(7.1)	
25-29	123 (24.8)	13(25.0)	100(25.3)	10(20.8)		9(18.4)	99(25.1)	15(28.9)		95(23.1)	28(33.3)	
30-34	159 (32.1)	16(30.8)	125(31.6)	18(37.5)		19(38.8)	123(31.1)	17(32.7)		135(32.8)	24(28.6)	
35+	153 (30.9)	19(36.5)	121(30.6)	13(27.1)		17(34.7)	118(29.9)	18(34.6)		127(30.8)	26(31.0)	
Parity	1.8 mean, 0.9 SD				0.2134				0.8585			0.2352
0	233 (47.0)	30(57.7)	186(47.0)	17(35.4)		25(51.0)	186(47.1)	22(42.3)		200(48.5)	33(39.3)	
1	159 (32.1)	15(28.9)	124(31.3)	20(41.7)		16(32.7)	126(31.9)	17(32.7)		130(31.6)	29(34.5)	
2+	104 (21.0)	7(13.5)	86(21.7)	11(22.9)		8(16.3)	83(21.0)	13(25.0)		82(19.9)	22(26.2)	
GDM					**0.0233**				0.6635			0.8362
No	353 (71.2)	29(55.8)	292(73.7)	32(66.7)		37(75.5)	277(70.1)	39(75.0)		294(71.4)	59(70.2)	
Yes	143 (28.8)	34(44.2)	104(26.3)	16(33.3)		12(24.5)	118(29.9)	13(25.0)		118(28.6)	25(29.8)	
Ethnicity					**0.0064**				0.2405			0.2151
Non-Hispanic	314 (63.3)	43(82.7)	241(60.9)	30(62.5)		27(55.1)	250(63.3)	37(71.2)		266(64.6)	48(57.1)	
Hispanic	182 (36.7)	9(17.3)	155(39.1)	18(37.5)		22(44.9)	145(36.7)	15(28.9)		146(35.4)	36(42.9)	
Race					0.1227				**0.0423**			0.9020
White	269 (54.2)	32(61.5)	208(52.5)	29(60.4)		23(46.9)	212(53.7)	34(65.4)		225(54.6)	44(52.4)	
Black	27 (5.4)	4(7.7)	20(5.1)	3(6.3)		1(2.0)	25(6.3)	1(1.9)		23(5.6)	4(4.8)	
Asian	52 (10.5)	8(15.4)	42(10.6)	2(4.2)		7(14.3)	36(9.1)	9(17.3)		44(10.7)	8(9.5)	
Other/Mixed	148 (29.8)	8(15.4)	126(31.8)	14(29.2)		18(36.7)	122(30.9)	8(15.4)		120(29.1)	28(33.3)	

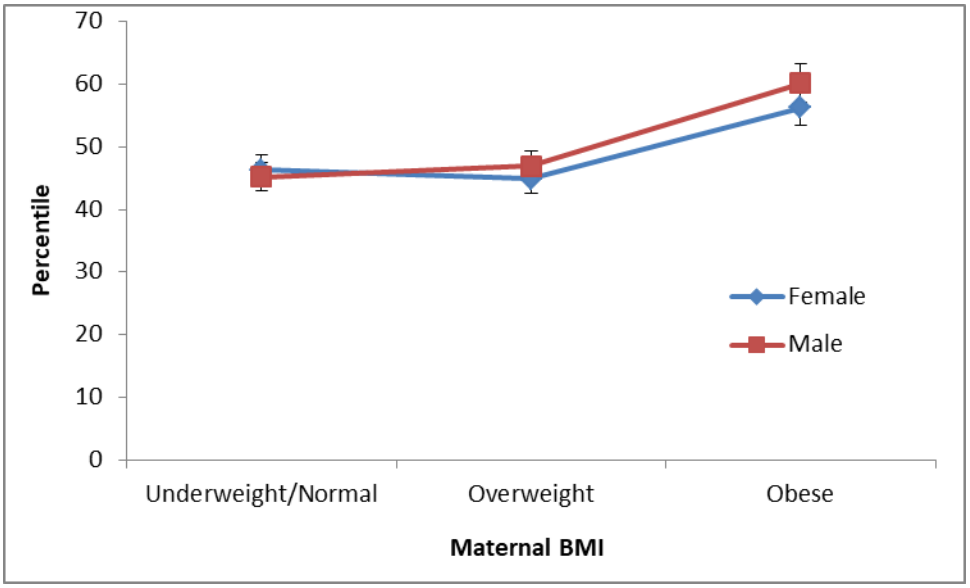

Figure 4-1 Gestational age-adjusted percentile of placental weight by maternal BMI stratified by female (p=0.0008) and male(p<0.0001) fetal sex. Note: p-values represent linear test for trend; y-axis range does not begin at zero.

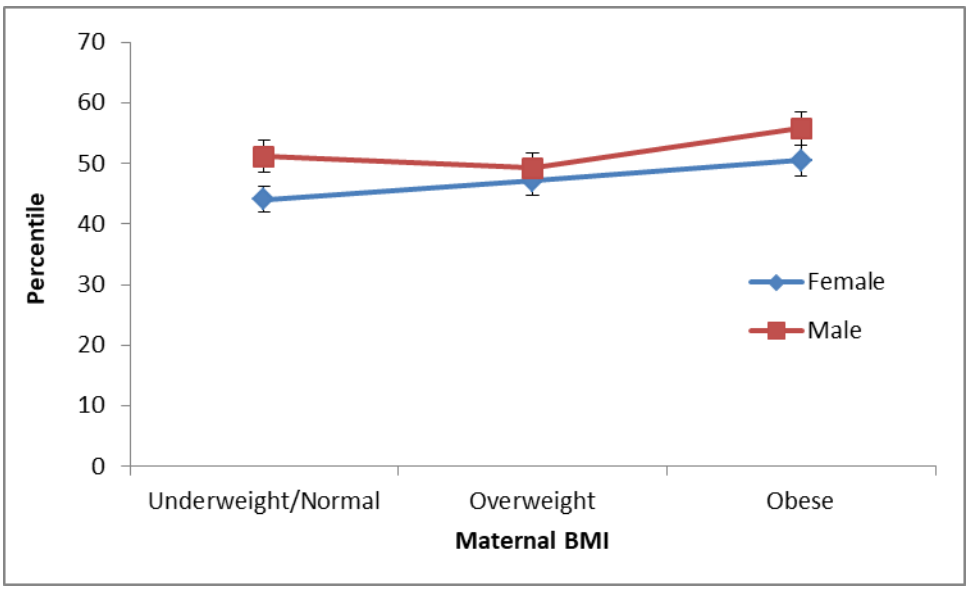

Figure 4-2. Gestational age-adjusted percentile of umbilical cord length by maternal BMI stratified by female (p=0.1032) and male (p=0.1681) fetal sex. Note: p-values represent linear test for trend; y-axis range does not begin at zero.

Table 4-3 Risk Ratios of Maternal BMI effect on Placental Health Outcomes by Fetal Sex, 2010-2015)

	Obesity*					
	Unadjusted RR			Adjusted RR		
Placental Health Outcomes**	**RR**	**95%CI**	**p-value**	**aRR**	**95% CI**	**p-value**
Female (n=469)						
LGA Placental Weight	**1.96**	**(1.02, 3.77)**	**0.0425**	1.42	(0.70, 2.88)	0.3285
LGA UC Length	1.50	(0.73, 3.09)	0.2699	1.05	(0.47, 2.34)	0.9118
Abnormal UC Insertion	0.53	(0.26, 1.05)	0.0669	0.62	(0.30, 1.29)	0.2020
Chronic Villitis	**2.03**	**(1.26, 3.27)**	**0.0036**	**1.79**	**(1.05, 3.04)**	**0.0313**
Male (n=496)						
LGA Placental Weight	**2.40**	**(1.32, 4.39)**	**0.0044**	**2.52**	**(1.33, 4.77)**	**0.0044**
LGA UC Length	**2.04**	**(1.14, 3.68)**	**0.0164**	**3.01**	**(1.56, 5.80)**	**0.0010**
Abnormal UC Insertion	1.01	(0.58, 1.78)	0.9671	1.03	(0.98, 1.09)	0.5026
Chronic Villitis	1.40	(0.85, 2.30)	0.1820	1.30	(0.77, 2.20)	0.3395

Model adjusted for maternal age, parity, ethnicity, and race; except for when analysis was stratified by fetal sex. Abbreviations: BMI – body mass index, RR – relative risk, aRR – adjusted relative risk, CI – confidence interval, LGA – large for gestational age, UC – umbilical cord; * Reference Non-Obese maternal BMI, ** Reference Non-LGA Placental Weight, Non-LGA UC Length, Normal UC Insertion, No Villitis

Table 4-4 Chronic Villitis by Placental Outcomes in Women with Maternal Obesity, 2010-2015 (n=287)

	Overall		Placental Weight				Cord Length			
			SGA	AGA	LGA		SGA	AGA	LGA	
	(n=287)		(n=18)	(n=228)	(n=42)		(n=18)	(n=233)	(n=36)	
Characteristics	N (%)		N (%)	N (%)	N(%)	p-value	N(%)	N(%)	N(%)	p-value
Overall			18(6.3)	228(79.4)	42(14.3)		18(6.3)	233(81.2)	36(12.5)	
Chronic Villitis						0.2913				0.1086
No	219	(76.3)	11(61.1)	176(77.2)	32(78.1)		15(83.3)	172(73.8)	32(88.9)	
Yes	68	(23.7)	7(39.9)	52(22.8)	9(22.0)		3(16.7)	61(26.2)	4(11.1)	
Female	140	(48.8)	12(8.6)	110(78.6)	18(12.9)		9(6.4)	118(84.3)	13(9.3)	
Chronic Villitis						0.3671				0.2927
No	102	(72.9)	7(58.3)	83(75.5)	12(66.7)		8(88.9)	83(70.3)	11(84.6)	
Yes	38	(27.1)	5(41.7)	27(24.5)	6(33.3)		1(11.1)	35(29.7)	2(15.4)	
Male	147	(51.2)	6(4.1)	118(80.3)	23(15.7)		9(6.1)	115(78.2)	23(15.7)	
Chronic Villitis						0.4894				0.3161
No	117	(79.6)	4(66.7)	93(78.8)	20(87.0)		7(77.8)	89(77.4)	21(91.3)	
Yes	30	(20.4)	2(33.3)	25(21.2)	3(13.0)		2(22.2)	26(22.6)	2(8.7)	

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Chapter 5

Discussion

With the growing epidemic of obesity among women in the US and across the globe, understanding this chronic condition's impact of the health of women during pregnancy is paramount. Pregnancy is a multifactorial process that depends on the balance of various systems. At the beginning of that process is the health status of the woman. Within days, the woman's body is interacting with the developing embryo and the exposure to her physiological environment begins playing a role. As the placenta develops along with the umbilical cord after fertilization, the maternal-fetal dyad begins to flourish. Therefore, a woman's health at conception and its impact on different aspects of the pregnancy is vital to understand. This dissertation was focused on providing a deeper insight into the effects a woman's pregravid maternal BMI and the amount of weight gained during pregnancy had on a specific aspect of the pregnancy process, the placenta, for the purpose of gaining a better understanding of how maternal exposures influence birth outcomes.

Results from Chapters 1-3 add to the growing knowledge of how the health of the woman at conception impacts the development and growth of the placenta with three key findings 1) Women with maternal obesity are more likely to have larger placentas, longer umbilical cords, and inflammation in the placental tissue compared to women without maternal obesity; 2) When evaluating the amount of weight gained during pregnancy, women with excessive weight gain have higher risks of longer umbilical cords compared to women with normal or insufficient weight gain, with the magnitude of that risk greater in pregnancies of male

fetuses; and 3) The effects of maternal obesity on the placenta and umbilical cord vary by the sex of the fetus, with the prevalence and magnitude of the risk varying depending on the outcome that is evaluated.

While previous research has investigated the effects maternal BMI has on various aspects of pregnancy, many times they are either laboratory focused assessing genetic or metabolic processes without the inclusion of demographics and/or macro characteristics of the mother or vice versa where observational studies assess various measureable confounders, but lack inclusion of biological markers or metabolic values. This dissertation aimed to merge these two types of studies, bridge the gap. These studies assessed the effects of demographic and clinical characteristics on histological and pathological outcomes with the ability to control for variability in the subjects on maternal variables such as age, parity, race and ethnicity, and GDM status.

Defining and implementing strategies to improve prenatal care and treatment for both the mother and the fetus with the goals to inevitably increase successful birth outcomes and ongoing health are key. The findings from these studies provide valuable insight into modifiable behaviors to which interventions can be focused and areas of surveillance by which high-risk pregnancies can be identified.

Maternal obesity and increased risk of abnormal placentas and umbilical cords

Chapter 1 titled *The effects of pregravid body mass index and gestational weight gain on indicators of placental health* assessed the effect of maternal BMI on placental weight, UC length, UC insertion and CV in a cohort of women treated within an academic care center. The findings suggested that high maternal BMI, after controlling for factors that confound both

maternal BMI and placental health, is associated with abnormal placental measurements. This supports previous research that maternal BMI, while a measureable exposure at conception, does have an effect on the pregnancy as well as the health of the fetus and mother both during gestation and after birth.[1-4]

Study 1 suggested that increases in maternal MBI, specifically obesity, increased the risk of large placentas, long umbilical cords and chronic villitis. Each of these placental outcomes has its own set of associated negative birth outcomes. Large placentas are correlated with increased birth weight.[5] LGA fetuses are more likely to experience delivery complications that put the lives of the infant as well as the mother at risk.[2] LGA infants also have increased risk of developing cardiovascular conditions as well as metabolic conditions such as T2DM later in childhood and even adulthood.[2,7] Long umbilical cords are more threatening during pregnancy and delivery due to the extra length increasing the risk of tangles and knots; all of which can complicate delivery. [8-10] Finally, chronic villitis is an indicator of inflammation in the placental tissue. The involvement of the placenta tissue reduces its functionality and if severe enough, can cause negative impacts of fetal development.[11] We built upon those findings to determine if GDM mediates the effect obesity has on placental weight. It has been repeatedly reported that GDM is associated to maternal BMI, with increases in BMI associated with increased risk of GDM.[12] We hypothesized that the effect obesity has on placental weight might be mediated by the environment of excess glucose created by GDM during gestation. Our findings didn't reject our hypothesis, but they were lower than expected. Additional analyses are needed to further define the mediating effect. More distinct definition of GDM could be used. Inclusion of patients that depended only on medicinal intervention to control GDM could provide a clearer picture of the mediating effect. On the other hand, there is mounting evidence that a mother's

diet during pregnancy has an effect on fetal development. Therefore, those patients controlling GDM with diet alone still might be valuable.

Abnormal umbilical cords and excessive gestational weight gain

Chapter 2 titled *Assessing the effect of gestational weight gain on placental health measurements* compliments the findings reported in Chapter 1. Gestational weight gain is influenced by various factors including pregravid BMI.[13] The suggested amount of weight a woman should gain during gestation is based on their BMI at conception. Women with obesity are counseled to gain fewer pounds than a woman with a BMI in the normal range. Interestingly, the results from Chapter 2 were not synonymous with the results from Chapter 1. While Chapter 1 reported abnormal placental and umbilical cord measurements due to the effects of pregravid obesity, Chapter 2 only found an effect of excessive weight gain on the length of the umbilical cord without an effect on placental size or inflammatory placental tissue. This conclusion implies that the BMI of the mother at conception affects placental outcomes differently than the amount of weight gained during gestation. The study did not find an association between gestational weight gain, no matter the initial BMI, and placental weight compared to normal weight gain; whereas pregravid obesity did have an effect on the size of the placenta compared to non-obese pregravid BMI. Similarly, Chapter 2 did not report an effect of gestational weight gain on the occurrence of CV in placental tissue, while Chapter 1 found an increase in CV in women with obesity at conception.

The growing base of knowledge would benefit from evaluating the effect of gestational weight gain by each level of maternal BMI to determine if the effects of weight gained during pregnancy varies depending on the woman's BMI at conception. Chapter 2 assessed GWG controlled for maternal BMI, but this might dampen an effect seen only within a specific

pregravid BMI cohort. While the modification of the effect by fetal sex was evaluated in Chapter 2, sample size limitations prevented further assessment by additional subgroups. Future studies might aim to estimate the effects of GWG on placental health with the goal of determining the impact maternal BMI has on the association.

Fetal sex modifies the effects of maternal obesity on the placenta

Chapter 4 titled *Fetal sex modifies the effect of maternal BMI on multiple indicators of placental health* aimed to enrich the findings of Chapter 2 by determining if fetal sex acts as a modifier of the effects reported in Chapter 2. In other studies, effects have been shown to vary depending on the sex of the fetus during pregnancy.[14,15] The goal of Chapter 2 was to evaluate a cohort of women overall to determine if a significant effect was present. Chapter 4 builds upon those results by stratifying by fetal sex. The findings suggest that fetal sex does modify the effects pregravid obesity has on placental development. The abnormalities affected by pregravid BMI in a pregnancy with a female fetus are not the same as found in a pregnancy with a male fetus. The placental outcomes affected in placentas of a female fetus are associated with inflammation, whereas the placental outcomes affected in placentas with a male fetus are associated with growth and development. This delineation of outcomes suggests that metabolic differences due to the sex of the fetus within the *in utero* environment could play a role. Placentas with a female fetus and affected by maternal obesity were at higher risk of inflammatory lesions such as CV compared to placentas with a male fetus also affected by maternal obesity. On the other hand, placentas with a male fetus and affected by obesity were at higher risk of larger placentas and longer umbilical cords compared to placentas with a female fetus also affected by maternal obesity.

Because of the lack of effect pregravid obesity has on the size of the placenta in pregnancy with a female fetus and the nature of CV to reduce growth, it was hypothesized that in placentas affected by maternal obesity CV would attenuate the growth effects on the placenta due to maternal obesity. Chapter 4 attempted to assess this hypothesis, but small sample size of women with maternal obesity and CV at delivery was a limiting factor. While significant findings were not reported, trends in the overall cohort of women with pregravid obesity and CV compared to those without CV are shown which could be used to generate more hypotheses. More than expected SGA placentas were in the cohort of women with CV and maternal obesity than those without CV. While this does not reject the null hypothesis, the question remains, if more patients available, would the trend be statistically significant? In other words, does CV attenuate the growth of the placenta in women with maternal obesity?

Interestingly, Chapter 3 reported longer umbilical cords in women with excessive gestational weight gain, but only in pregnancies with a male fetus. These results were controlled for pregravid BMI, therefore suggesting that excessive GWG, no matter the maternal BMI, is associated with an increased risk of longer umbilical cords. With the results of Chapter 4, new questions begin to surface. If excessive weight affects the length of the umbilical cord, does having pregravid obesity exacerbate that effect and in return increase the risk even greater in women with maternal obesity and excessive weight gain during pregnancy?

Strengths and Limitations

This dissertation did have limitations. Generalizability of our results may be limited due to the cohort of women analyzed in this dissertation. Our cohort represents a group of women who were more likely to receive prenatal care and deliver at a regional academic care center. An inclusion criteria for each of these studies was that each woman had to have a maternal weight

measurement during the first 14 weeks of gestation. If this initial measurement was missing, the woman was excluded from the studies which represented 17% of the initial cohort of possible subjects. Women without a weight measurement either during the first 14 weeks of gestation might represent patients with minimal prenatal care or access to prenatal care, which has been linked to poor birth and maternal outcomes at delivery. Women with reduce prenatal care miss the opportunities for counseling on best practices during pregnancy and the chance to be monitored and treated for clinical symptoms that could in turn reduce poor birth outcomes. In addition, the demographic makeup of the women with reduced prenatal care might disproportionately distributed among maternal age, race, ethnicity, and SES status. Based on the descriptive statistics of the initial cohort considered for analysis prior to applying the inclusion and exclusion criteria, the final study cohort had fewer Hispanic patients than the initial cohort as well as less patients with an "Other" race categorization. Procedures were implemented to overcome this obstacle as much as possible by accessing medical records to abstract missing information when available. While race and ethnicity were reported for this cohort of women, the status was based on self-reported data in the medical records not a standardized collection tool. Therefore, there could be variability within each category based on one's self recognition. This dissertation was unable to evaluate SES status which has been shown to be associated with obesity, reduced prenatal care and poor birth outcomes.

Nevertheless, the studies in this dissertation also have numerous strengths. Our sample size was large, allowing for stratification by variables of interest, including BMI, fetal sex, ethnicity, race, and GDM. The ability to abstract information from both the Perinatal Repository and medical records in EPIC provided the opportunity to create a robust data set that allowed for the incorporation of various potential confounders, both demographic and clinical. This begins

to strengthen all findings by bridging gaps due to inability to incorporate both demographic and clinical characteristics. In addition, all placentas were evaluated using a standardized system developed by a single pathologist specializing in placental assessment, providing consistent results and reducing interobserver variability.

Conclusion

Women with obesity prior to becoming pregnant have increased risks of abnormal placenta growth and development when they do become pregnant. Women of child-bearing age that are considering becoming pregnant would benefit with even small decreases in weight prior to conception. With the rise in prevalence of obesity in the US, increasing our understanding of its health consequences is a public health mission. In terms of obstetrics, obesity's impact on fetal and placental development, along with delivery and birth, is continually being revealed. The maternal-fetal dyad is an intricate system that begins days after fertilization therefore the health status of the woman is vital not only throughout gestation but at the moment of conception. Insight into areas that focused counseling and awareness would be most effective is key in reducing poor birth outcomes. Clinical cognizance of how maternal BMI and gestational weight gain affects placental outcomes allows for directed surveillance and timely interventions when necessary. Lastly, discussing the significance of weight not only during pregnancy but prior to pregnancy could be an efficient way to modify behaviors and reduce the health burden on both the mother and the infant.

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