Pregnancy Body Mass Index and its Relationship with Maternal and Fetal Health Outcomes in Rural Appalachia

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Abstract

Increased weight gain or obesity in pregnancy has been identified as a risk factor for multiple adverse maternal and child health outcomes that are of large public health concern. Maternal obesity can also complicate obstetric care by preventing identification of ultrasound markers or decreasing available fetal cell-free DNA fraction for routine genetics studies.

The study analyzes the pregnancy, birth, and early childhood data from 995 mother-child pairs in the COHRA2 study to assess maternal body mass index and its associations with pregnancy risk factors and childhood health outcomes in this population.

We found that women and children in our sample were similar to the United States population in the prevalence of obesity (36%) during pregnancy and had an increased prevalence of gestational diabetes mellitus (8%) as a pregnancy complication. We observed a low overall frequency of congenital birth defects (1.9%), and no significant difference in frequency between infants born to obese versus nonobese mothers. Additionally, we were able to replicate findings from studies in other populations, observing that there was a higher rate of Cesarean delivery for women who gave birth to macrosomic (>4000 grams) infants ($p=0.003$), and that children who were macrosomic at birth were more likely to be obese in childhood and were younger at the time of their first tooth eruption, illustrating that obesity is a risk factor for adverse childhood outcomes even past the neonatal period. Lastly, we found significant differences between the Pittsburgh and West Virginia participants in our study, demonstrating that urban and rural populations have

significant sociodemographic differences (particularly in rates of obesity ($p=2.19x10^{-5}$), frequency of Cesarean delivery ($p=0.005$), and time to first tooth eruption ($p<0.0001$) that are important considerations when developing public health interventions.

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Preface

I would like to acknowledge the COHRA research team and the Center for Craniofacial and Dental Genetics for their invaluable skill and knowledge. It has been a privilege to learn research skills with this dynamic, dedicated team of individuals who work incredibly hard at what they do. Your commitment to excellence in your field will have a lasting impact on my professional career, your research participants, the community you serve, and the body of research you support. Further, thank you for the opportunity to contribute to your team in my work position, gaining experience for my public health practicum, and allowing me to bring a genetic counselor's perspective to the table in my thesis research.

Also, my deepest gratitude goes to my thesis committee for their support, leadership, and wisdom in developing this thesis project. I thank them for their participation in my endeavors, helping me develop an appreciation for research, and for their flexibility during the effects of the COVID-19 pandemic. To Robin Grubs, Andrea Durst, and Candace Kammerer: I expect I will only continue to value your mentorship and guidance exponentially as I grow into my professional career. Thank you for continually challenging and inspiring me.

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1.0 Introduction

Fetal development is impacted by complex interactions between genetic predispositions and exposures from nutrition, maternal health conditions, and maternal lifestyle influences (Institute of Medicine (US) Committee on Nutritional Status During Pregnancy and Lactation, 1990; Julihn et al., 2009). The prevalence of overweight and obesity are rising worldwide (Rahman et al., 2015; Sommer et al., 2014; Wang et al., 2019) and maternal overweight and obesity pre-pregnancy or during pregnancy raise concern for adverse maternal and fetal outcomes. A growing body of evidence suggests that obese women who have excessive weight gain during pregnancy experience the highest risk for adverse pregnancy outcomes (Durie et al., 2011; Ferrari & Siega-Riz, 2013). Figure 1 demonstrates the breadth of adverse outcomes that have been associated with maternal overweight, obesity, and/or increased weight gain during pregnancy.

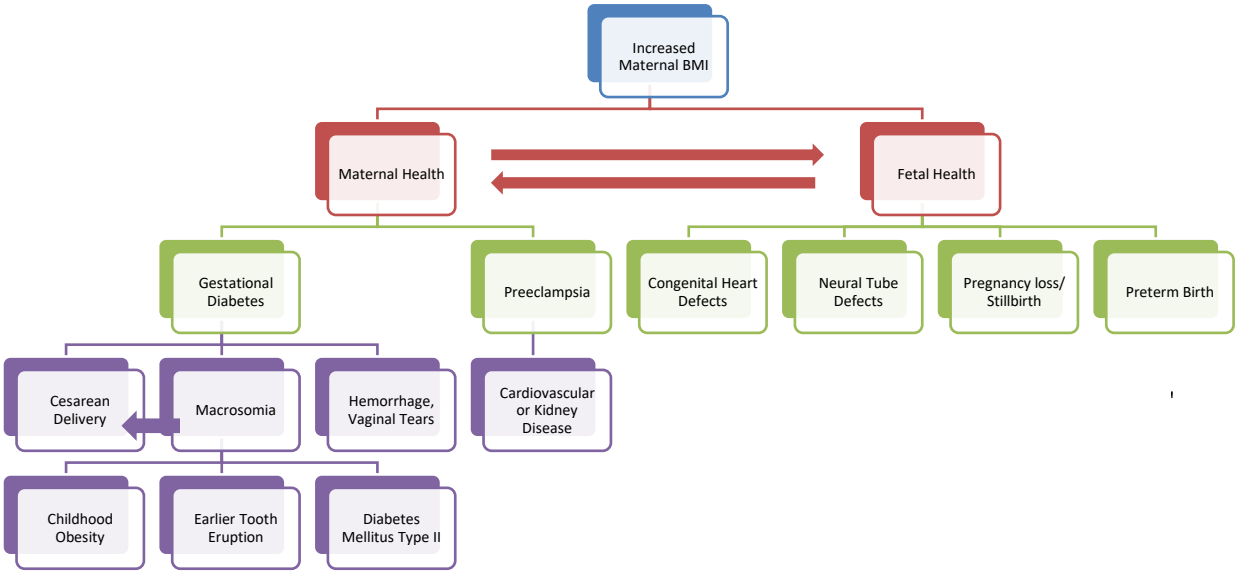


Figure 1: Maternal Overweight and Obesity Associations Cited in Literature

Past the neonatal period, few studies to date have analyzed the long-term consequences associated with an increased pregnancy BMI. One reason may be the complex relationship of maternal weight gain with other pregnancy factors, socioeconomic factors, nutritional status and profile, and/or chronic maternal disease. Obesity risk genes have also been shown to be associated with gestational weight gain (Beysel et al., 2019; Groth & Morrison-Beedy, 2015; Lawlor et al., 2011; Martins et al., 2016; Meng et al., 2018; Sharp et al., 2017).

Due to the interplay of these various factors and their downstream effects on children's health and behaviors, it is of interest to know if there is any relationship between maternal BMI and health outcomes in their children past the neonatal period. Socioeconomic and geographic influence are important when considering a public health intervention, and differences that may exist between groups from different regions of the world should be considered.

Genetic counselors interact with pregnant women for a variety of indications and are an integral part of many expectant mothers' obstetric care. As a resource for exploring the risks and benefits of genetic tests for a variety of indications, genetic counselors also counsel about risks for congenital birth defects – some of which, such as neural tube defects, omphalocele, and congenital heart defects, have been shown to be associated with higher maternal BMI (Racusin et al., 2012) in the absence of genetic etiologies. Genetic counselors also often discuss with women how the results of Noninvasive Prenatal Testing (NIPT) can be impacted by increased maternal weight. However, it is prudent to consider maternal weight as more than just a modifier on test outcome, and that an overweight or obese woman may have specialized pregnancy considerations for which she should be assessed (Beysel et al., 2019; Bianchi et al., 2018; Sharp et al., 2017). There is evidence that detection methods for fetal anomalies are more limited by maternal obesity (Racusin et al., 2012), owing to the inability to visualize some anatomical structures (both fetal and

maternal) because of the maternal body fat. This limitation to screening is placing these women at a higher risk for a complicated pregnancy that is mismanaged due to lack of detection of fetal malformation.

The goal of the current study is to investigate the role of maternal BMI on outcomes in children, specifically on adverse health outcomes that are preventable. Children aged 2-5 years living in Appalachia have a childhood caries (cavities) rate that is 144% of the national average (Wen et al., 2017), taken from data gathered from the Center for Disease Control's 1999-2004 National Health and Nutrition Examination Survey (National Institute of Dental and Craniofacial Research, 2018). "Implementing preventative measures against childhood diseases, such as early childhood caries, caries and other metabolic conditions (Carolina Un Lam et al., 2016)" has been cited as support for investigating the complex relationship between prenatal risk factors and eruption timing of the first tooth. This study of obesity will be a novel investigation into the role of a global health problem's impact on a population that is vulnerable to phenomena that perpetuate health disparities. With this study, we aim to contribute further evidence in favor of prenatal healthcare providers – including genetic counselors – and public health professionals supporting healthy weight as a risk-reducing intervention.

1.1 Factors Affecting Rural Appalachia

Children living in Appalachia demonstrate poorer oral health and elevated rates of caries earlier in life compared with the national averages (Neiswanger et al., 2015). Research from the University of Pittsburgh's Center for Oral Health Research in Appalachia (COHRA) has previously illustrated the various unique demographic characteristics of the population in Rural

Appalachia. Appalachia is a region of the eastern United States, covering parts of northern Mississippi, Alabama, Georgia, and reaching up to western New York – roughly corresponding to the Appalachian mountain range. Due to the mountainous terrain, the region is largely rural, and its populations have been historically geographically isolated. Individuals living in Appalachia have historically experienced higher levels of poverty and food insecurity (Neiswanger et al., 2015).

Women enrolled in the "Factors Contributing to Oral Health Disparities in Appalachia" study (COHRA2 and COHRA Smile) live in West Virginia and Western Pennsylvania and delivered a singleton pregnancy at 36 weeks or more between 2012 and 2019. They identify as either White (Non-Hispanic or Latino) or African American. Women are English speaking and non-immunocompromised.

We have also previously reported significant differences within our study population between the populations from Pittsburgh and West Virginia. In 2015, the 727 participants in COHRA2 were assessed for demographic characteristics via telephone and in-person interview as part of their study visit. The following table (Table 1) is adapted from Neiswanger et al. (2015) and shows the areas on the questionnaires where women in Pittsburgh and West Virginia significantly differed in their responses. These include personal, household, and social behavioral demographics. Pregnant participants from Pittsburgh had more education, higher income, greater employment, more dental insurance and private medical insurance, self-reported better health, had fewer children in the household, less food anxiety, and drank more and smoked less than participants in West Virginia (Neiswanger et al., 2015).

Table 1: Demographic Differences in the COHRA2 Study

Demographic differences between Pittsburgh and West Virginia residents enrolled in the COHRA2 study. Adapted from Neiswanger et al. 2015					
Personal Demographics		Household Demographics		Social Behavior	
Education Level	P <0.00001	Additional people in household	P=0.002	Drank alcohol any time up until 2nd trimester	P <0.00001
Ever employed	P = 0.0008	Additional Children	P=0.0002	Smoked 3 months prior to pregnancy through 2nd trimester	P=0.02
Current employment status	P <0.00001	Worried food would run out in the past year	P=0.001		
Type of Medical Insurance	P<0.00001	Food did run out in the past year	P=0.001		
Having Dental Insurance	P<0.00001	Household Income	P<0.00001		
Self-report general health	P=0.002				

The COHRA study population represents a unique subset of the United States population. For the aforementioned reasons, the COHRA studies aim to identify genetic, lifestyle, metabolic, and socioeconomic factors that contribute to the oral health in this population. The robust data set gathered by COHRA investigators also contributes information about maternal-fetal wellness, due to the longitudinal nature of the study. There may be overlap in the risk factors that contribute to healthy pregnancy outcomes and oral health disparities; and there is reason to consider that this population may also be at risk for adverse neonatal outcomes.

2.0 Literature Review

2.1 Pregnancy BMI

While the highest proportion of obesity in women worldwide is in those 55 and older, trends in adult age-standardized obesity prevalence show that more rapid weight gains occur between ages 20-40 years (Ng et al., 2014). This age range encompasses the majority of women of child-bearing age. It is estimated that 33% of women in the United States are obese, and that the number of obese pregnancies is 1.1 million, representing 34.9% of pregnant women (Chen et al., 2018).

Maternal body mass index (BMI) during pregnancy is a known risk factor for multiple adverse outcomes for both the mother and the developing fetus (Chen et al., 2018) and is a growing public health problem worldwide (Beysel et al., 2019; Bianchi et al., 2018). The exact biological mechanism for how increased BMI has an adverse effect on a pregnancy is not known. One hypothesis is that obesity increases adipocyte number or pelvic soft tissue mass. Adipocyte abundancy may be a cause of excessive inflammatory responses, influencing the development of gestational diabetes; while pelvic soft tissue could cause narrowing of the birth canal and warrant cesarean delivery (Rahman et al., 2015). Conversely, negative control studies have investigated the postnatal environmental and genetic confounders in the relationships between maternal and paternal adiposity and child outcomes; these studies have found only limited support for the effects of maternal adiposity on offspring adiposity, and it is worth investigating whether maternal adiposity has other, intrauterine effects on the developing fetus (Sharp et al., 2017).

Overall, higher body mass index during pregnancy is considered a risk factor for both mother and fetus. We will briefly highlight research on both excess weight gain during pregnancy and excess weight in general while pregnant. Both states are risk factors for adverse health outcomes.

2.2 Weight Gain During Pregnancy

Fetal growth accounts for approximately 25% of all weight gain during pregnancy, while expansion of maternal tissues (uterine and mammary tissue mass, blood volume, extracellular fluid, and fat stores) accounts for approximately two-thirds of the total gain (Institute of Medicine (US) Committee on Nutritional Status During Pregnancy and Lactation, 1990). High gestational weight gain has been associated with long-term maternal weight retention, risk for type II diabetes, and other obesity-related disorders for mothers later in life (Hales, 2017; Mannan et al., 2013; Nehring et al., 2013; Sharp et al., 2017; Walker et al., 2011).

Goldstein et al. completed a systematic review and meta-analysis of over 1 million pregnant women which found that 47% had gestational weight gain greater than Institute of Medicine (IOM) recommendations and 23% had gestational weight gain less than IOM recommendation. Gestational weight gain that is greater than guideline recommendations, compared with weight gain within recommended levels, was associated with higher risk of adverse maternal and infant outcomes. Table 2 includes the IOM's 2009 guidelines for healthy gestational weight gain based on World Health Organization (WHO) categories of BMI (Goldstein et al., 2017).

Table 2: IOM Gestational Weight Gain Guidelines

Gestational Weight Gain Guidelines		
Category	**WHO BMI Category (%)**	**IOM Suggested Gestational Weight Gain (kg)**
Underweight	<18.5	12.5-18
Normal	18.5-24.9	11.5-16
Overweight	25-29.9	7-11
Obese	30 or greater	5-9

The 2009 IOM guidelines also included recommended rates of weight gain per week for the second and third trimesters, defined by weight category: 1-1.3 pounds for underweight, 0.8-1 pound for normal weight, 0.5-0.7 pound for overweight, and 0.4-0.6 pound for obese (Institute of Medicine (US) and National Research Council (US) Committee to Reexamine IOM Pregnancy Weight Guidelines, 2009). Limited weight gain or even net weight loss have not been shown to have an effect on small-for-gestational age neonates in obese women and this change may even improve perinatal outcomes (Durie et al., 2011). In the Pregnancy, Infection, and Nutrition (PIN) Study at the University of North Carolina, 78% of women reported gaining weight outside the current provider recommendations (Ferrari & Siega-Riz, 2013).

A 2004 study by Kabiru et al. followed 5131 women during singleton pregnancies. The normal weight women who had an increase in BMI category between the start of pregnancy and the end had an associated higher rate of gestational diabetes, failed induction, lacerations, and postpartum infection. In overweight women, increased rates of preeclampsia and operative vaginal deliveries were observed. Obese women had higher rates of chorioamnionitis (an infection usually associated with prolonged labor), failed induction, and Cesarean deliveries (Kabiru & Raynor, 2004).

Durie et al. studied a large cohort of women to analyze the effects of excessive second- and third-trimester weight gain on pregnancy outcomes. They showed that in their cohort of women

with singleton pregnancies, there was an increased odds of labor induction and admission to the neonatal intensive care unit if women were of normal weight and had excessive rates of weight gain (Durie et al., 2011). Additionally, women in all weight classes who had excessive rates of second-or third-trimester weight gain had an increased odds of having a cesarean delivery (Durie et al., 2011).

Usta et al. found that 366 newborns, representing 8.6% of newborns in their study were macrosomic at birth (weighing at least 4000 grams). In the macrosomic vs. non-macrosomic infants, maternal age, parity, pre-pregnancy BMI and gestational weight gain were higher in the group of mothers who had macrosomic infants (Usta et al., 2017). Mothers had cesarean deliveries more often when fetuses were macrosomic, and male fetuses were more likely to be macrosomic (Usta et al., 2017).

2.3 Overweight or Obesity and Pregnancy

Ovesen et al. showed that in a cohort of Danish women who had singleton pregnancies from 2004-2010 (a total of 369,347 women), those who were considered overweight or obese had significantly increased risks of several adverse pregnancy outcomes. These outcomes included those that affected the mother, the delivery, and the neonate. Outcomes primarily affecting the mother included hemorrhage, gestational diabetes mellitus (GDM), thrombosis, and preeclampsia. Those that affected the delivery included planned or emergency Cesarean section, or shoulder dystocia with vaginal delivery; those that primarily affected the neonate included macrosomia (defined as weighing >4500g), low Apgar score, and stillbirth (Ovesen et al., 2011). Appendix A illustrates their findings of how increased BMI increases the risk of the aforementioned pregnancy

outcomes. In summary, they found a significant association between maternal weight and each of these outcomes and identified that the odds of the adverse outcomes increased with increasing maternal weight.

2.3.1 Gestational Diabetes Mellitus

Gestational diabetes mellitus occurs when a woman develops insulin resistance and inadequate insulin secretion for the first time during pregnancy (ACOG, 2017). Maternal insulin is known to be the primary growth hormone responsible for fetal development (Usta et al., 2017). Because women with GDM subsequently pass more glucose to the fetus than normal, the risk for fetal macrosomia is increased. This complicates pregnancies by putting women at risk for labor difficulties, necessitating a cesarean delivery, hemorrhage, or severe tears to the vagina. The prevalence of GDM in the United States is estimated at 5.6% (Deputy et al., 2018); with the prevalence being average in Pennsylvania (5.5%) and elevated in West Virginia (7.2%) (Deputy et al., 2018).

Some women are at an increased risk of developing GDM, including those who had GDM in a previous pregnancy, have high blood pressure, are of certain ethnicities, or are overweight or obese (ACOG, 2017; Sommer et al., 2014). Developing GDM also increases a woman's risk to develop hypertension and preeclampsia. Women who develop GDM are at an increased risk to develop type II diabetes later in life (Sommer et al., 2014; Stubert et al., 2018).

In addition to macrosomia, babies born to women with GDM are at an increased risk to have breathing problems, jaundice, low blood sugar at birth, as well as an increased risk of being overweight, obese, and developing diabetes in childhood (ACOG, 2017).

2.3.2 Preeclampsia

Preeclampsia is a condition that occurs secondary to chronic hypertension during a pregnancy, typically in the third trimester. It is characterized by signs of organ failure secondary to hypertension, including proteinuria, low platelet count, abnormal kidney and/or liver function, pain in the upper abdomen, vision changes, fluid in the lungs, or severe headaches (ACOG, 2019). Women who are at a moderate to high risk for preeclampsia include those who have previously had preeclampsia, have diabetes mellitus, or other conditions such as hypertension, kidney disease, and autoimmune conditions, those who are African American, over 35 years of age, are pregnant for the first time, or have a body mass index over 30 (ACOG, 2019). If a woman experiences preeclampsia, it may mean that the fetus needs to be delivered preterm, which poses risks to the baby including mortality. In addition, women with preeclampsia have an increased risk of developing cardiovascular and/or kidney disease later in life (especially those who deliver a preterm baby). Preeclampsia can also lead to eclampsia (seizures) and HELLP syndrome, a medical emergency characterized by hemolysis, elevated liver enzymes, low platelet count, and an increased risk of hepatic internal bleeding (ACOG, 2019).

2.3.3 Pregnancy Loss

Pregnancy loss, by miscarriage (20 weeks gestation or fewer) or intrauterine fetal death (more than 22 weeks gestation) are risks that have an increased association with maternal overweight during pregnancy. Miscarriage occurs at an increased rate in women who are obese compared with those who are of normal weight; additionally, recurrent miscarriage has been shown to be more common in obese women than in women of normal weight (Stubert et al., 2018).

Stubert et al. continue that there is an increased prevalence of intrauterine fetal death (IUFD) in obese women compared to normal-weight women, caused by abnormal function of the placenta combined with arterial hypertension (Stubert et al., 2018). Liu et al. found that when mothers were overweight or obese, infants had a higher risk of still birth (OR 1.27, 1.81 respectively, 95% CI) (Liu et al., 2016). Further, Yao et al. found that maternal obesity was associated with approximately 25% of stillbirth between 37 and 42 weeks gestation (Yao et al., 2014).

2.3.4 Preterm Birth

Preterm birth – both spontaneous and medically indicated – has a higher prevalence among obese mothers and contributes to unfavorable neonatal outcomes. In a study of 12,950 deliveries, obese and overweight women were at an increased risk to have a large-for-gestational-age (LGA) delivery compared with normal BMI women in the study (Ehrenberg et al., 2004), demonstrating that each risk factor contributes independently to the risk of a macrosomic infant.

2.3.5 Macrosomia

Macrosomia occurs when there is excessive fetal weight gain (typically defined as weighing more than 4000-4500 grams). The prevalence of fetal macrosomia varies among ethnic groups, but occurs in approximately 6-10% of all newborns worldwide (Usta et al., 2017) and is estimated at 7% in the United States (Martin et al., 2018). Women who experience excessive gestational weight gain or are obese are more likely to deliver a macrosomic baby (Stubert et al., 2018). As previously noted, hyperglycemia associated with GDM can lead to fetal macrosomia. Macrosomia is also more likely in pregnancies where women have a higher pre-pregnancy BMI

(Agudelo-Espitia et al., n.d.). Fetal macrosomia, maternal obesity, and excessive weight gain during pregnancy are associated with childhood-onset obesity. Women who give birth to macrosomic babies are at an increased risk of infection, postpartum hemorrhage, prolonged labor, high degree perineal tears, anesthetic accidents, and thromboembolic events (Usta et al., 2017), and macrosomic fetuses are at higher risk of birth complications and/or defects, including perinatal asphyxia, meconium aspiration, clavicular fracture, brachial plexus injury, and shoulder dystocia (Chatfield, 2001). Children who were macrosomic-at-birth are also at increased risk to develop hypertension, obesity, and type II diabetes mellitus (Usta et al., 2017). Though maternal gestational diabetes is a risk factor for macrosomia, the majority of macrosomic fetuses are born to women without gestational diabetes mellitus and further, no identifiable risk factors for macrosomia (Usta et al., 2017). There is no known utility to dietary restriction during pregnancy for its effect on fetal macrosomia (Chatfield, 2001).

2.3.6 Shoulder Dystocia

Shoulder dystocia is a delivery complication that occurs when the baby's shoulders become lodged in the pelvis as a result of being proportionately too large for the birth canal. This complication is twice as likely to occur when maternal BMI is greater than 35 (Ovesen et al., 2011) Shoulder dystocia is likely a downstream effect of the culmination of other risk factors, including gestational diabetes, gestational age, and macrosomia. Shoulder dystocia increases the risk of maternal postpartum hemorrhage, vaginal lacerations, anal tears, and uterine rupture (Ovesen et al., 2011).

2.3.7 Cesarean Delivery

Cesarean delivery procedures are performed over 1 million times each year in the United States (Yuan et al., 2016). Though it is a common procedure that reduces risk of maternal and fetal mortality there are risks associated with cesarean delivery. Maternal risks include infection, hemorrhage (Keag et al., 2018), an increased risk of mortality, 5-fold increased risk of cardiac arrest, 3-fold risk of hysterectomy and puerperal infection, and 2-fold risk of thromboembolism (Yuan et al., 2016). Pregnancy after a cesarean delivery is associated with an increased risk of miscarriage, stillbirth, placenta previa, placenta accrete, and placental abruption (Keag et al., 2018). Compared to children born via vaginal delivery, children delivered via cesarean delivery experience respiratory complications at an increased frequency, including asthma, childhood wheeze, dermatitis and allergy (Keag et al., 2018; Yuan et al., 2016); these children also experience higher rates of other adverse health events throughout childhood and later in life (Yuan et al., 2016). A 2016 meta-analysis of a large cohort consisting of over 22,000 individuals found that cesarean birth was associated with a higher risk of obesity (Yuan et al., 2016). Further, analysis of siblings in the study who were born by discordant modes of delivery (cesarean or vaginal) found that the odds of obesity were 64% higher for individuals born by cesarean delivery versus their siblings born by vaginal delivery (Yuan et al., 2016). A 2018 meta-analysis found that cesarean delivery was associated with an increased odds of childhood obesity continuing throughout childhood up to early adulthood (Keag et al., 2018).

2.3.8 Birth Injury

A population-based study from the Swedish Medical Birth Registry found that maternal BMI was associated with adverse neonatal outcomes (Blomberg, 2013). The authors discuss that admission to a neonatal intensive care unit is based on regional clinical guidelines and therefore not standard, however, review of the literature and findings from their own population study indicated that morbid obesity in mothers conferred a significantly increased risk for many and varied adverse neonatal outcomes, which all require NICU admission for treatment (Blomberg, 2013). Table 3 lists these adverse outcomes based on information from the Swedish Medical Birth Registry from 1998-2008.

Table 3: Birth Injuries and Maternal Morbid Obesity in Sweden

Birth Injuries Strongly Associated with Maternal Morbid Obesity in Sweden			
Central Nervous System	**Peripheral Nervous System**	**Skeletal**	**Other**
Intracranial laceration	Erb paralysis	Fracture to skull, femur, other long bones, clavicle, etc.	Birth asphyxia
subdural hemorrhage	Klumpke paralysis		Aspiration syndromes/ pulmonary hemorrhage
cerebral hemorrhage	Phrenic nerve paralysis		
intraventricular hemorrhage	Brachial plexus nerve injuries		Bacterial sepsis of the newborn
subarachnoid hemorrhage			
cerebral edema			Convulsions of the newborn
brain damage			
cranial nerve/ spinal cord injury			Feeding problems
			hypoglycemia

2.4 Influence of Maternal Weight on Clinical Genetics Studies

The ability of noninvasive prenatal testing (NIPT) to detect fetal trisomic chromosomes relies on the proportion of fetal DNA (fetal fraction) present in a sample of maternal plasma. If fetal fraction is below 4%, NIPT cannot provide a result as to the detection of a fetal trisomy. Studies show that fetal fraction is significantly associated with maternal weight, such that fetal fraction decreases with increasing maternal weight (Ashoor et al., 2013; Stubert et al., 2018). Figure 2 illustrates findings from a 2013 study by Ashoor et al. which found that increased maternal weight decreased fetal fraction during a critical time period for genetics studies.

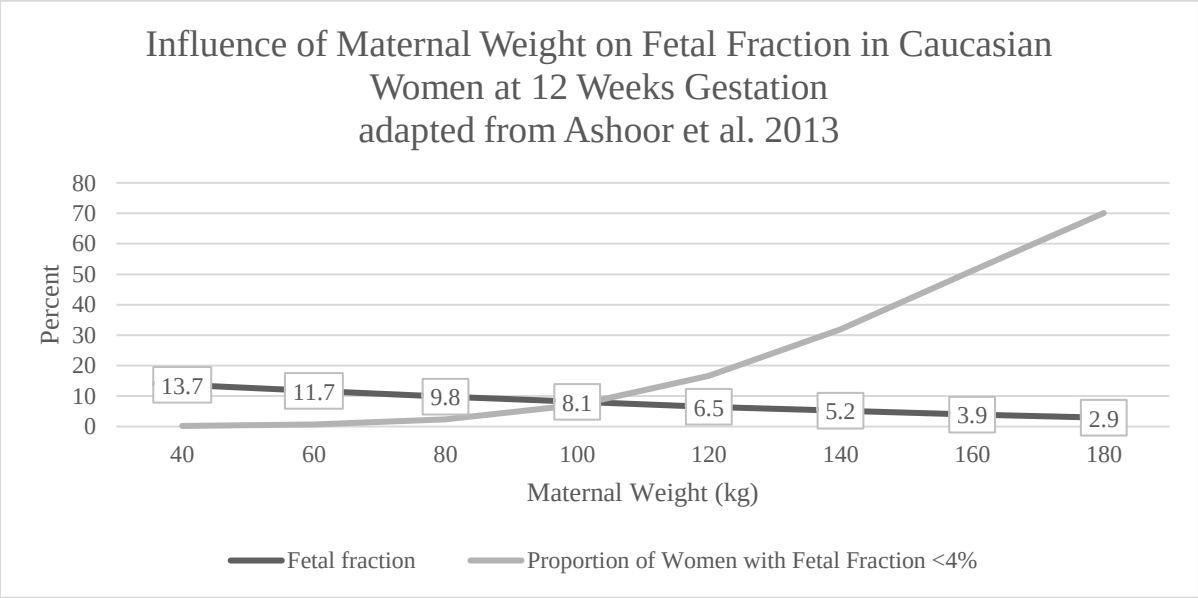


Figure 2: Influence of Maternal Weight on Fetal Fraction

This study found that the greatest contribution of fetal fraction was provided by maternal weight (Ashoor et al., 2013). Assessment at 11-13 weeks of gestation is ideal for detection of aneuploidies in genetics, but also for predicting many pregnancy complications including preterm

birth, preeclampsia, and fetal growth restriction. This study found that the greatest contribution to fetal fraction was provided by maternal weight.

Obese women have a higher rate of miscarriage with normal chromosome studies (euploid miscarriage). A 2014 study by Boots et al. investigated whether there was a significantly increased frequency of euploid miscarriage in obese women with recurrent early pregnancy loss (REPL). 372 women with REPL (578 miscarriages) were included in the study; REPL was defined as two or more pregnancy losses at less than 10 weeks of gestation, and at least one ultrasound-documented miscarriage with chromosome results was required. The findings from this study showed that 58% of obese women's miscarriages were euploid, while 37% of nonobese women's miscarriages were euploid (Boots et al., 2014). Further, the two groups (obese and nonobese) did not significantly differ in the frequency of endocrine factors, autoimmune factors, or endometritis (Boots et al., 2014), eliminating possible confounding factors in this analysis. This study indicates that maternal obesity may be a risk factor for early pregnancy loss regardless of chromosome abnormalities in the fetus, especially with a history of miscarriage.

Diabetes and hyperglycemia are risk factors for neural tube defects (NTDs) (Racusin et al., 2012). NTDs also show dose dependence, where maternal overweight is a risk factor for neural tube defects and obesity is a risk factor specifically for spina bifida and anencephaly (OR for NTDs in obese women: 1.85, 95% CI (Racusin et al., 2012)).

2.5 Influence of Maternal Weight on Childhood Health

Obese pregnant women are at increased risk of adverse health outcomes such as gestational diabetes and preeclampsia. Their offspring are at increased risk for birth defects, macrosomia, and

morbidity associated with subsequent childhood obesity (Kabiru & Raynor, 2004; Madsen et al., 2012). Development of human primary dentition begins at the end of the fifth week of gestation (Ntani et al., 2015). Timing of primary incisor eruption has been shown to have a strong heritability of over 70% (Ntani et al., 2015). Various processes can occur during critical periods of fetal development that can result in lifelong effects on health; many factors associated with advanced dental development are also associated with primary dentition dental caries (Ntani et al., 2015). Regardless of pre-pregnancy BMI, it has been reported that up to 62% of pregnant women have an excess in gestational weight gain (Durie et al., 2011). This excess weight gain has been shown to have implications for maternal health, neonatal health, as well as continued health of the child.

2.5.1 Birth Defects

Maternal BMI has previously been shown to be associated with other adverse health outcomes in children. One example is illustrated in a study of congenital heart defects. Of infants born between 1992 and 2007 in Washington, U.S.A., 14,142 were identified as having a congenital heart defect (CHD). After excluding those with a known chromosome abnormality or those whose mothers had diabetes mellitus, birth records from these infants found that those born with CHD were more likely to have an obese mother (Odds ratio: 1.22, 95% CI: 1.15-1.30); and furthermore, this association was found to increase with increasing maternal BMI (Madsen et al., 2012). This finding was replicated by the Atlanta Birth Defects Risk Factor Surveillance Study which reproted that pregnancy BMI was an independent risk factor for congenital heart defects (Racusin et al., 2012). Evidence from these studies implicates obesity as a teratogenic factor in a developing pregnancy.

Similarly, the Pregnancy Health Interview Study (Slone Birth Defects Study) indicates a relationship between maternal weight and neural tube defects. Mothers with high dietary glycemic index (DGI) or dietary glycemic load (DGL) have been shown to have a 1.5 and 1.9 times greater odds, respectively, of having a pregnancy complicated by a neural tube defect (NTD) (Yazdy et al., 2010). These results implicate maternal obesity and/or diabetes as risk factors for neural tube defects. One hypothesis for this phenomenon is that, in the setting of hyperglycemia, there is an increased rate of apoptosis in neural tube cells of the growing fetus, leading to a neural tube defect (Madsen et al., 2012). Longitudinal and cross-sectional population-based data indicate that a higher BMI is correlated with lower serum folate levels, regardless of folate intake. Since the 1990s, folate supplementation has not been shown to reduce the incidence of NTDs in fetuses of obese women (Racusin et al., 2012).

2.5.2 Higher Birth Weight and its Associated Childhood Morbidities

Tyrrell et al. (2016) studied data from newborns in the Early Growth Genetics Consortium to determine whether there was genetic evidence of a causal relationship between maternal BMI and birth complications. They studied maternal risk factors hypothesized to increase fetal growth including BMI, fasting glucose level, gestational or type II diabetes, triglyceride level, high-density lipoprotein cholesterol (HDL-C) level, and adiponection level. Additionally, they analzyed maternal factors hypothesized to decrease fetal growth including hypertension and lower vitamin D levels. Single-nucleotide polymorphisms were selected to represent each trait based on previous evidence (Tyrrell et al., 2016) to create a weighted genetic score for the maternal traits, which was analyzed for association with birth weight and neonatal leanness. Overall, higher birth weight and ponderal index (lower neonatal leanness) had significant associations with maternal

BMI, fasting glucose, and type II diabetes genetic scores (Tyrrell et al., 2016). The results from this study indicate that there is evidence for a causal relationship between maternal glucose levels (glycemia) and higher birth weight via increased placental glucose transfer and fetal insulin secretion (Tyrrell et al., 2016). It is important to consider these results as they represent a complex multifactorial link between maternal genetic risk scores for a pregnancy outcome of higher birth weight.

A systematic review by Falvigne et al. reported that treatment of GDM was effective in reducing the rates of macrosomia, preeclampsia, and shoulder dystocia. The risk of fetal macrosomia should be considered during prenatal care for pregnant women with pre-gestational or gestational diabetes mellitus. In this study, maternal age parity, pre-pregnancy BMI, and gestational weight gain of mothers were significantly higher in mothers of macrosomic infants than in controls. Additionally, rates of cesarean deliveries were significantly higher, and maternal age over 35 years tripled the risk of fetal macrosomia (Usta et al., 2017).

The finding that macrosomia is associated with earlier eruption of primary teeth is consistent across many studies and populations (Delgado et al., 1975; Warren et al., 2016; Wu et al., 2019). A 2019 study by Wu et al. analyzed 1296 Chinese mothers aged 20 to 41 in their first trimester of pregnancy. They found that 5.23% (58 of 524) infants had macrosomia, defined as weighing more than 4000g at birth. Macrosomia was found to be significantly associated with earlier eruption timing of the first primary tooth (ETFPT) (Wu et al., 2019). Macrosomic-at-birth infants, on average, had their first tooth eruption at 5.91 months of age, which was significnatly lower than the 6.82 months for the mean of all infants in the study. This is consistent with findings that higher birth weight is associated with earlier tooth emergence, and prematurity and low birth

weight seem to be associated with delayed tooth emergence (Delgado et al., 1975; Warren et al., 2016).

Similarly, Garmash et al (2019) found that the highest burden of (dental) caries were recorded in children who were macrosomic-at-birth with well-balanced intrauterine development, with intrauterine obesity and increased body length, or with intrauterine obesity and average body length. Additionally, they found that children born with fetal macrosomia had long narrow faces and high palates more frequently than normosomic-at-birth children from their study (Garmash 2019).

2.6 Lifestyle Considerations

It is important to note that the relationship proposed here between maternal BMI, infantile macrosomia, and childhood morbidities such as obesity and early tooth eruption should not be considered linear. Most likely these conditions are enmeshed with other risk factors for poorer oral health outcomes in children. Studies have shown that maternal lifestyle and socioeconomic risk factors – notably obesity and smoking – are associated with poorer dental health in children, including increased rates of caries development in both primary and permanent teeth (Julihn et al., 2009). A link between childhood obesity and dental caries has been described in the literature, but little is known about any relationship between maternal (over)weight and dental caries in children. One hypothesis is that maternal overweight, via overnutrition during prenatal and perinatal development, has long-term effects on children – specifically, their continued eating habits and appetite regulation have impact on caries development (Julihn et al., 2009).

2.7 Tooth Eruption

Tooth eruption in children is a tightly regulated process that involves physiological interactions within the oral cavity as well as a genetic element of regulation (Carolina Un Lam et al., 2016). Primary tooth formation and eruption are both sequentially controlled and require interactions pre- and postnatally. Tooth eruption demonstrates a relatively high (at least 70%) heritability (Carolina Un Lam et al., 2016) and so variation in the timing of tooth eruption outside of genetic influence appears multifactorial and is not as well understood. When there are disturbances to the patterns of tooth formation and/or eruption, there are several possible etiologies: physical/structural pathologies include dental follicle, root, and alveolar bone; systemic pathologies include aberrations of cellular, molecular, and/or genetic origin (Carolina Un Lam et al., 2016).

At the molecular level, the dental follicle and parathyroid hormone-related protein regulate metabolic changes in the alveolar bone to stimulate tooth eruption (Declerck et al., 2007). Overweight and obesity in childhood have been associated with early eruption of primary teeth, as has childhood diabetes mellitus – suggesting the importance of metabolic processes on the timing of tooth eruption. Because these processes happen simultaneously, it is likely that prenatal, perinatal, and postnatal factors all have an inter-related influence on the timing of a child's first tooth eruption.

3.0 Manuscript

3.1 Background

Increased body mass index during pregnancy is a risk factor for adverse maternal (Kabiru & Raynor, 2004; Stubert et al., 2018) and fetal (Ehrenberg et al., 2004; Stubert et al., 2018; Yao et al., 2014) outcomes. Children born to overweight or obese mothers can experience higher rates of congenital birth defects (Madsen et al., 2012; Racusin et al., 2012; Yazdy et al., 2010) and/or adverse health outcomes later in childhood (Julihn et al., 2009; Keag et al., 2018). Obesity is a complex disease of public health importance that affects pregnancy (Keag et al., 2018; Stubert et al., 2018) and can be onset in childhood. Childhood onset obesity is associated with other childhood comorbidities, including juvenile diabetes, earlier tooth eruption, and poorer dental health outcomes (Delgado et al., 1975; Garmash, 2019; Ntani et al., 2015; Warren et al., 2016; Yuan et al., 2016). The population in rural Appalachia represents a uinque subset of the United States that is subject to higher rates of adverse health outcomes due to being historically isolated geographically, as well as sociodemographically disadvantaged (Neiswanger et al., 2015).

The current study will further the current understanding of the relationship between BMI and pregnancy outcomes, including childhood health, in Appalachia. The study uses a subset of data gathered from women and children participating in the University of Pittsburgh and West Virginia University's longitudinal research study, COHRA2: Factors Affecting Oral Health in Disparities in Appalachia.

3.1.1 COHRA2: Factors Affecting Oral Health Disparities in Appalachia

The Center for Oral Health Research in Appalachia (COHRA) study, Factors Affecting Oral Health Disparities in Appalachia, is a longitudinal study aiming to identify genetic, microbial, and environmental determinants of oral health in northern Appalachia (Neiswanger et al., 2015). The study enrolls pregnant women to follow over the course of their pregnancy and child's development. Data are collected on mothers' general and oral health, households' and individuals' environments, and infants' general and oral health (Neiswanger et al., 2015). During study visits, participants provide answers to surveys about their health, participate in dental assessments, and provide saliva samples for microbial analysis and DNA extraction. The data used for the following analyses were obtained from the surveys and measurements taken at participants' COHRA2 study visits.

Over the course of time, participants in the COHRA2 study have provided information relevant to their child's growth and development. Now that the study has completed visits through age two for all mother-child pairs in the initial (Caucasian women) arm of the study, it is pertinent to assess data on childhood growth and development in the first two years of life. We now have enough data to compare our study population to the trends observed throughout the United States and other parts of the world, i.e., to assess whether the population of Appalachia – who are being assessed for risk factors for general and oral health outcomes – are also a population that has higher risks associated with pregnancies.

3.1.2 Eligibility Criteria

Women were eligibile for enrollment in COHRA2 if they lived in the United States, and were Caucasian, generally healthy, English-speaking, not infected with tuberculosis or immunocompromised, and were pregnant. Singleton pregnancies less than 29 weeks gestation were eligible for enrollment in the study.

3.1.3 Study Aim

The goal of this study is to assess whether there is a significant relationship between maternal weight and adverse maternal, neonatal, or childhood health outcomes. The specific aims for the study are as follows.

3.1.3.1 Specific Aims

1. Obtain a data set of mother-child pairs participating in the COHRA2 study to define as the study cohort.
2. Obtain, from a set timepoint in the exisiting dataset, BMI measurements, maternal health information, birth measurements, and early childhood health information to define variables for study, along with basic demographic variables.
3. Define relationships between maternal BMI and dependent variables of interest; analyze whether there is a significant relationship between BMI and any of the cited risk factors in the COHRA2 participants.

4. Identify statistically significant demographic differences between cohorts from West Virginia and Pennsylvania to comment on sociodemographic differences in the study cohort.

3.2 Methods

3.2.1 Institutional Review Board

The COHRA2 study has been approved by the University of Pittsburgh and the West Virginia University IRBs. Written informed consent was obtained prior to beginning any research procedures. This study used data from the COHRA2 study, and no additional IRB approval was required.

3.2.2 Data Collection

Data were collected from participants during study visits and included a mix of self-reported information and measurements taken by a research coordinator. The visits occurred at set time points and were recorded via SNAP online surveys. For this study, information was selected from several different visits. Information about mothers was obtained from the initial study visit, also known as the prenatal visit. Pregnancy body mass index was calculated using mothers' weights and heights measured at the prenatal study visits, and gestational age calculated by their reported due dates. Using recommendations from professional obstetric guidelines (Government of Canada, 2014; Institute of Medicine (US) and National Research Council (US)

Committee to Reexamine IOM Pregnancy Weight Guidelines, 2009), high BMI alone is a risk factor at any point in pregnancy and this study did not control for gestational age in determining whether it influenced BMI. Due to lack of data on rates of weight gain by trimester that are representataive of the U.S. population (Institute of Medicine (US) Committee on Nutritional Status During Pregnancy and Lactation, 1990), we did not adjust for gestational age in determining BMI status.

Information about pregnancy complications, labor, delivery, and newborn biometrics (length, weight, and head circumference) were obtained from the birth visit or first postnatal visit. Data on childhood growth parameters (height and weight) were obtained at one, two, and three-year visits.

In Pittsburgh, a participant's fourth visit occurs 2-3 weeks after the child's first primary tooth erupts, rather than at a set time point for a child's age. Approximate ages of children at the time of first primary tooth eruption were obtained from phone interview and visit records.

3.2.3 Data Categorization and Cleaning

The Institution of Medicine's guidelines for healthy weight gain during pregnancy are outlined in a chart of suggested weight gain per week of gestation (see Table 1). This chart was used to categorize whether women were within a healthy weight category at the time they entered the study. Using this chart, women were categorized into one of four BMI categories: BMI 1 were below the recommended range (less than 18.5), BMI 2 were within the recommended range (18.5-24.9), BMI 3 were in the overweight range (25.0 – 29.9), and women in BMI 4 were obese (30.0 or greater).

Mother-child pairs were excluded from the study if there was not a pregnancy BMI or weight available for the mother, the baby was born prematurely, or if measurements for baby's birth height and weight were unavailable.

Infants were classified as macrosomic-at-birth if they weighed at least 4.00 kilograms. Children's weight was classified at subsequent visits. The CDC's National Center for Health Statistics Data Tables for Girls or Boys Weight-for-length charts were used to identify the percentile for growth of each child at their one, two, and three-year visits. Children identified as being at or above the 95^{th} percentile for growth were classified as obese (Centers for Disease Control and Prevention National Center for Health Statistics, 2019).

3.2.4 Data Analysis

Data were visualized and tested using Microsoft Excel. For all tests, a significance level of $\alpha=0.05$ was set. A two-tailed Z-test was utilized to compare the proportions of mothers in each BMI category by site, such that any statistical significance in a proportion of women in one BMI category at a given site could be identified.

The prevalence of pregnancy obesity in our study sample was compared to the general population of the United States. A 2018 meta-analysis of maternal obesity by Chen et al. utilized free-access databases from the World Health Organization to determine the rate of obesity in pregnancy and the approximate number of obese pregnant women at any given time. Their methods determined that the rate of obesity in pregnancy in the United States is 34.9% (Chen et al., 2018). This proportion was used as a comparison in a one proportion Z-test to determine the significance of obesity prevalence in our study sample.

Prevalence statistics reported by the CDC (Deputy et al., 2018) were used to compare the occurrence of gestational diabetes mellitus in our sample to that in the general population. A one-proportion Z-test was utilized to identify whether there was statistically significant difference in prevalence.

The proportion of Cesarean deliveries performed was compared two ways: the proportion of West Virginia mothers versus Pittsburgh mothers who had Cesarean deliveries, and the proportion of normosomic versus macrosomic infants delivered via Cesarean. Both comparisons were performed using two-tailed Z-tests to analyze proportions.

The proportion of macrosomic infants born to women in each BMI category was compared to the collective proportion of macrosomic infants born to women in the other three categories. A two-tailed Z test to compare proportions was used to determine any significant differences.

Pregnancies that resulted in a child with a congenital birth defect were analyzed for any pregnancy complications. Children with identified genetic and/or syndromic conditions were excluded from the subset, such that the analysis only included children with a seemingly idiopathic congenital defect. Retrospective analysis of the pregnancy complications was performed to identify any risk factors related to the birth defect, including preeclampsia, GDM, overweight or obesity, and preterm labor. A chi-squared goodness-of-fit test was used to test for significant differences in the distribution of birth defects across BMI categories.

3.3 Results

Overall, there were 1009 participants enrolled in the COHRA2 study for whom data was available. After excluding participants for whom data on pregnancy weight or birth weight of the

baby was not available, 995 mother-child pairs were defined as the study sample. 545 pairs (54.7%) were enrolled through the Pittsburgh study arm and 450 (45.3%) were enrolled through the West Virginia study arm. 532 babies were male (53.5%) and 462 were female (46.5%).

BMI: Figure 3 shows the distribution of pregnant women in each BMI category by recruitment site (West Virginia or Pennsylvania).

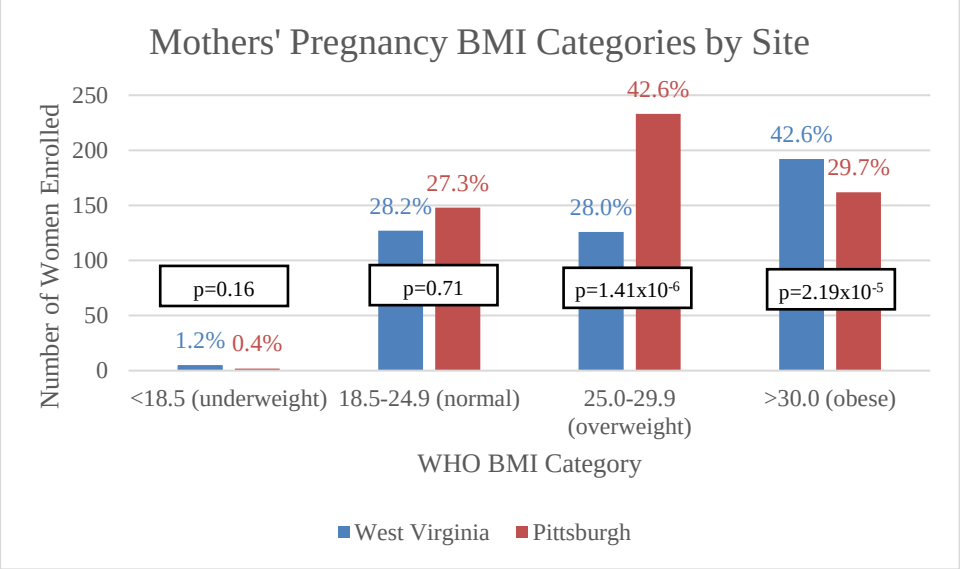


Figure 3: Distribution of Pregnancy BMI in Pittsburgh and West Virginia

Most women were overweight or obese. The overall rate of pregnancy obesity in our study sample (35.58%) was not higher than the general United States population (34.90%, p=0.65). There were no significant differences in the proportions of underweight (p=0.16) or normal weight (p=0.71) women enrolled at each site. A significant increase was identified in the proportion of overweight women enrolled in Pittsburgh ($p=1.41x10^{-6}$). Likewise, a significant increase was identified in the proportion of obese women enrolled in the West Virginia site ($p=2.19x10^{-5}$). Figure 4 summarizes the results from relationships tested in the study.

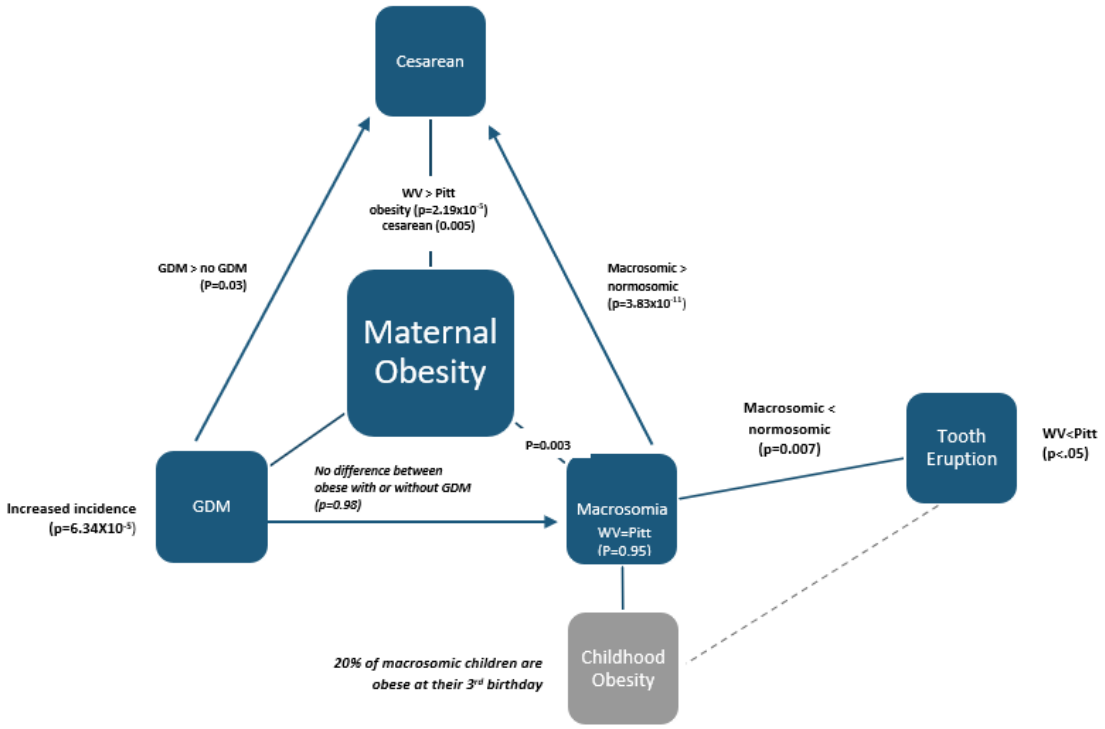


Figure 4: Relationships Between Maternal Obesity and Health Outcomes

This figure demonstrates proposed relationships from the observations made in this study. Maternal obesity was associated with an increase in macrosomia and Cesarean delivery. Gestational diabetes occurred at an increased rate from the general population and was associated with higher rates of Cesarean deliveries. Macrosomia occurred at similar rates in obese women with or without GDM. Macrosomic children had earlier tooth eruption. West Virginia study participants were more often obese, had more Cesarean deliveries, and had earlier tooth eruption. Rates of macrosomia and first tooth eruption may warrant further investigation into factors contributing to childhood obesity.

Delivery: Mode of delivery was reported for 990 babies in the study. Two hundred seventy-five babies were born via Cesarean delivery, comprising 27.78% of the study sample. 144 babies in West Virginia (32%) and 131 babies (24%) in Pittsburgh were reported as being delivered by Cesarean section. The difference in proportion of babies delivered via Cesarean was significantly different (p=0.005), wherein a greater proportion of West Virginian women had a

Cesarean delivery. Figure 5 illustrates the proportion of Cesarean deliveries distributed across the four maternal BMI categories.

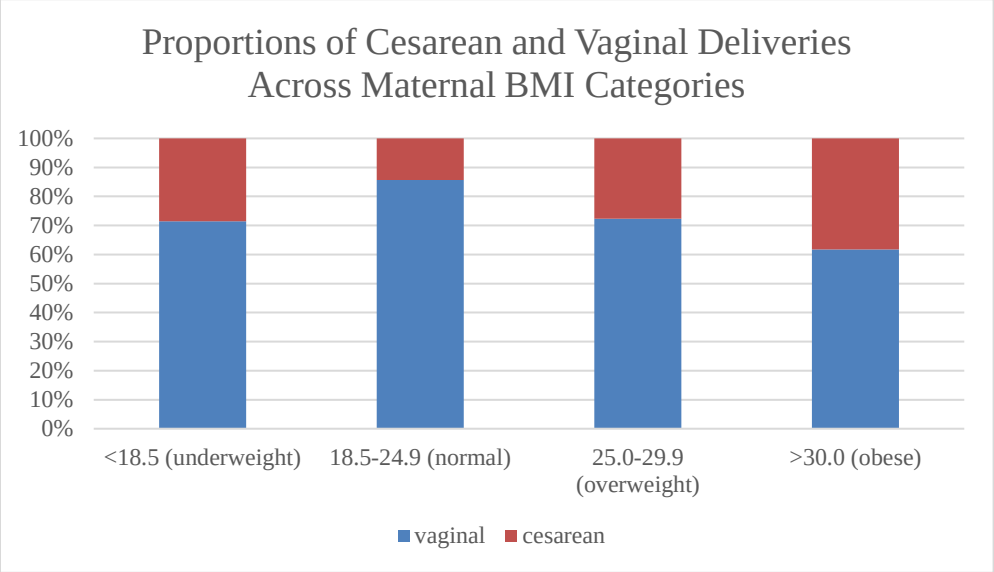


Figure 5: Cesarean and Vaginal Deliveries in COHRA2

There was a significant difference in the distribution of Cesarean deliveries across BMI categories ($p=2.398x10^{-33}$). No significant difference was found between the Cesarean delivery proportion for women in the "underweight" BMI category compared to the other three categories combined ($p=0.96$); and similarly, no significant difference was found between the proportions of babies born via Cesarean delivery in the "overweight" category compared to the proportion of Cesarean deliveries in the other categories ($p=0.95$). However, significantly more Cesarean deliveries were found in the obese study sample ($p=4.5x10^{-8}$) and significantly fewer for the normal weight study sample ($p=6.25x10^{-9}$).

Macrosomia: Figure 6 shows the proportion of macrosomic infants born to women in each BMI category at both West Virginia and Pittsburgh sites.

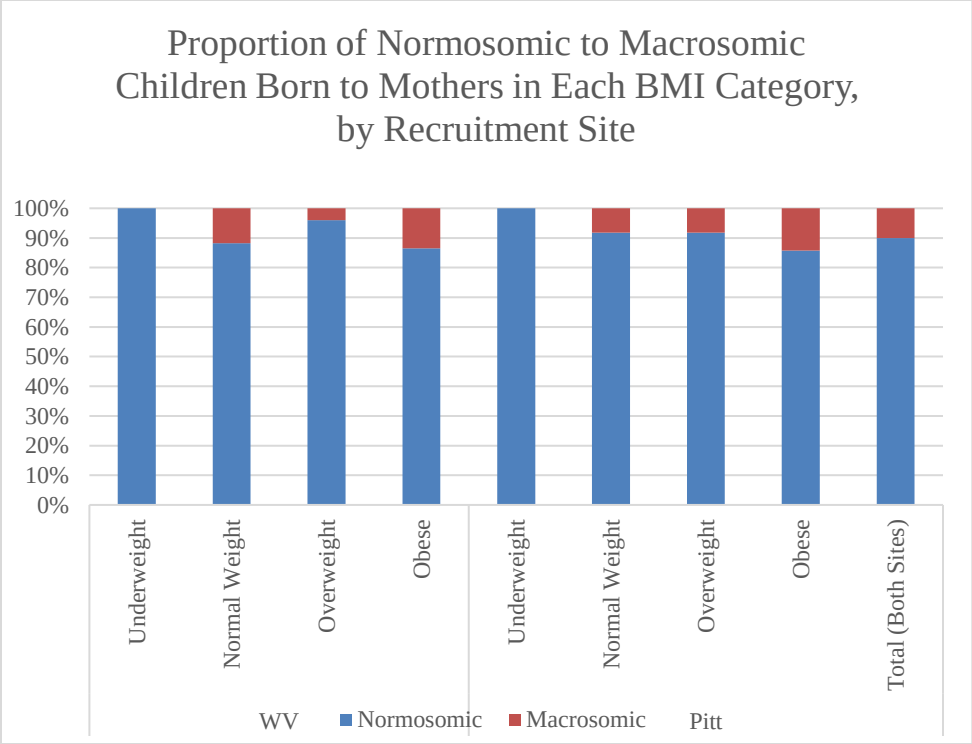


Figure 6: Normosomic and Macrosomic Infants Born to Women in Each BMI category

One hundred (100) babies were classified as macrosomic, comprising 10.1% of the study sample. There was no difference in the proportion of infants that were macrosomic between West Virginia (10.22%) and Pittsburgh (9.93%) (p=0.95). No women who were in the underweight BMI category at either site gave birth to a macrosomic infant. No significant difference was found between the macrosomia proportion for women in the "underweight" BMI category compared to the rest of the women (p=0.375); likewise, no significant relationship was found when comparing the macrosomia proportion in the normal BMI group to the other women (p=0.106). A significant difference in the proportion of macrosomic infants born to women whose BMI was categorized as overweight was observed compared to the macrosomia proportion in other women (p=0.008) and the most significant difference was seen in the proportion of macrosomic infants born to obese women compared to other women (p=0.003).

Gestational Diabtes Mellitus: Eighty-one women in our study reported gestational diabetes mellitus (8.14%). A one-proportion Z-test revealed that this prevalence (8.1%) was different from the prevalence of gestational diabetes mellitus in caucasian women in the U.S. general population (5.3%, reported by Deputy et al. 2016) ($p=6.34x10^{-5}$). 50 of 720 vaginal births were pregnancies complicated by GDM (6.94%). A total of 31 out of 275 Cesarean births were complicated by GDM; a two tailed Z-test for proportions revealed a significant difference in proportions of women with GDM who eventually had a Cesarean delivery and those who had a vaginal delivery (p=0.03).

Because there was a notable difference in the prevalence of GDM in our study, we repeated the prior test excluding women whose pregnancies were complicated by GDM. The results showed that there was no signficant difference in the proportion of macrosomic infants born to obese mothers with or without GDM (p=0.98).

Prenatal Risk Factors: Pregnancies resulting in Cesarean deliveries were assessed for prenatal risk factors. The collection of risk factors are summarized below in Table 4. Numbers that are highlighted represent categories in which the number of women who developed the condition exceeded the number of women who were identified as being at risk for the condition.

Table 4: Reported Risk Factors in COHRA2 Pregnancies

Risk Factor	**Number of Women Reporting**	
Risk for High Blood Pressure Reported	75	7.5%
• Diagnosed in 1st Trimester	21	2.1%
• Diagnosed in 2nd Trimester	27	2.7%
• Diagnosed in 3rd Trimester	167	16.8%
Diabetes	45	4.5%
Gestational Diabetes	33	3.3%
• Diagnosed in 1st Trimester	24	2.4%
• Diagnosed in 2nd Trimester	26	2.6%
• Diagnosed in 3rd Trimester	81	8.1%
Preeclampsia	64	6.4%
Ruptured Membranes	0	0%
Risk for Preterm Delivery	86	8.6%

Figure 7 illustrates observed risk factors in the study that resulted in 5 mother-child pairs experiencing three risk factors (GDM, Cesarean delivery, and macrosomia) for later adverse health outcomes. All five of these mothers were obese. Of these five, two pregnancies were also complicated by a risk for preterm labor and one by hypertension and risk of preterm labor. Of the 33 Cesarean deliveries to macrosomic infants, three pregnancies were complicated by hypertension and risk of preterm labor.

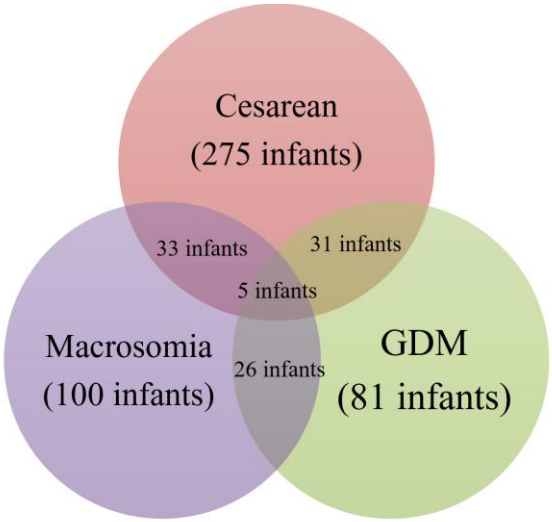


Figure 7: Overlapping Risk Factors Observed in COHRA2 Pregnancies

Congenital Defects: Data on congenital birth defects was collected for newborns. After excluding defects associated with genetic or syndromic conditions, 19 newborns were reported to have a congenital birth defect. (1.90% of the study sample). While sample sizes were too small to perform statistical analyses with enough power, the distribution of birth defects across maternal BMI categories is displayed in Figure 8. A list of the birth defects reported can be found in Appendix C.

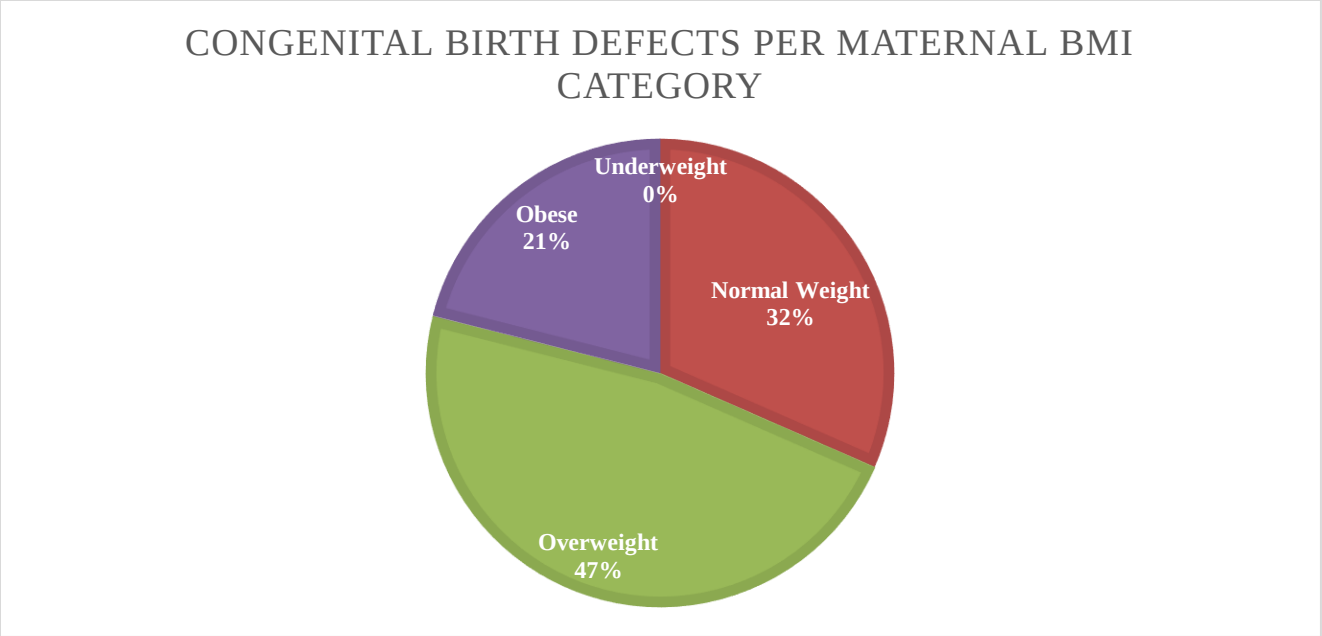


Figure 8: Congenital Birth Defects Reported in COHRA2

Fifteen infants had a congenital heart defect. Of these 15 infants, only one had a mother that developed gestational diabetes mellitus during pregnancy (6.67%). Six of the remaining 14 infants were born to mothers with normal BMI; an additional 6 were born to overweight mothers, and 2 were born to obese mothers. This distribution across BMI categories is not different than expected (p=0.36) given the BMI distribution of women in the study.

Childhood Obesity: Thirty-five children that were macrosomic at birth were above the 95th percentile (obese) on CDC growth charts at their first birthday. One-year data were unavailable for 16 macrosomic-at-birth infants, therefore this number represents 35.00% of macrosomic-at-birth children and an overall 2.51% of children in the study sample. Thirty-eight children that were macrosomic at birth were above the 95th percentile on growth charts at their second birthday. Twenty children who were macrosomic at birth were above the 95th percentile on growth charts at their third birthday.

Tooth Eruption: Data were available on the time of first tooth eruption for 526 children from Pittsburgh and 312 children from West Virginia (838 children total). The mean time to first tooth eruption in Pittsburgh was 32.64 weeks. The mean time to first tooth eruption in West Virginia was 27.47 weeks. A two-sample Z-test for means revealed a significant difference ($p<0.0001$). The mean time to first tooth eruption in normosomic infants was 31.39 weeks, while the mean in macrosomic infants was 29.77 weeks. Again, a two-sample Z-test for means revealed a significant difference ($p=0.007$).

3.4 Discussion

The results of this study show that more overweight women in Pittsburgh and more obese women in West Virginia are enrolled in COHRA2 study, comprising the majority of the study sample. The majority of Pittsburgh mothers were overwieght, suggesting that there may be pre-pregnancy differences in BMI between women from the two sites, which may be due to the socioeconomic differences (Neiswanger et al., 2015). The proportions of overweight women in Pittsburgh and obese women in West Virginia were equal (42%). Though the relationships tested in this study (and others (Bianchi et al., 2018; Ovesen et al., 2011; Sharp et al., 2017; Tyrrell et al., 2016; Usta et al., 2017)**)** indicate that obesity is a risk factor, the fact that 29% of women from Pittsburgh were obese should not be discounted. Differences in mean gestational age at the time of measurement were not considered in this study and may have influenced why fewer women from Pittsburgh were classified as obese. Studies observed relationships indicating that there is a dose-dependant relationship between maternal weight and fetal outcomes (Ovesen et al., 2011; Racusin et al., 2012). The women who were overweight may still be at risk if the risk is due to

pregnancy weight gain, and these women should also be counseled about the risks associated with increased maternal weight during prengancy.

West Virginia mothers had significantly more Cesarean deliveries than Pittsburgh mothers, and obese women from both sites combined for a significant majority of cesarean deliveries. Likewise, significantly more macrosomic infants were born to obese mothers. Associations between maternal obesity and both Cesarean delivery and macrosomia are observed in our study sample, which is consistent with the previously published data (Keag et al., 2018; Ovesen et al., 2011; Usta et al., 2017; Yuan et al., 2016).

The increased rate of Cesarean deliveries in West Virginia is well-documented. An initiative started in 2011 by the West Virginia Perinatal Partnership, West Virginia Chapter of the March of Dimes, the West Virginia Health Care Authority, and the West Virginia Health Statistics Center aimed to address the growing rate of Cesarean deliveries among nulliparous women. Their research showed that 35.1% of nulliparous West Virginian women delivered via Cesarean in 2009 (West Virginia Perinatal Partnership & March of Dimes, West Virgina Chapter, 2014). The beginning of this initiataive coincided with the beginning of recruitment for COHRA2. A yearly analysis or more recent data collection may find a smaller difference in the rate of Cesarean deliveries between these two sites, with West Virginia hospitals effecively implementing these interventions (West Virginia Perinatal Partnership & March of Dimes, West Virgina Chapter, 2014).

Of note, there was no significant difference in the number of macrosomic infants born in West Virginia and Pittsburgh. Because of this, the difference in the rate at which Cesarean deliveries were performed is of interest. We have previously discussed that there was a significant difference in the proportion of women from West Virginia that were obese. It is possible that the

women in the West Virginia arm of the study experienced more pregnancy complications that warranted Cesarean deliveries. Of course, it is also possible that there are differences in institutional threshold for performing Cesarean delivery. Most women in the Pittsburgh arm of the study delivered their babies at Magee Womens Hospital of University of Pittsburgh Medical Center (UPMC). Women in West Virginia are recruited from throughout the state and, as a group, may not have experienced access to a similarly standardized decision. Reasons for Cesarean delivery also exist that were not captured in this data set, including (but not limited to) having had a Cesarean delivery with a previous pregnancy.

We saw that there was a significant increase in the rate of Cesarean deliveries for women who experienced GDM. Additionally, we observed that there was not a significant difference in the rate of Cesarean births between obese women who did or did not have GDM, implying that obesity is associated with Cesarean delivery whether or not the increase in BMI leads to GDM.

The data from our study do not support an increased chance for congenital birth defects associated with maternal obesity. Though literature supports an increased risk of congenital heart defects or spina bifida with increasing maternal weight, our study indicates that in this sample, birth defects are distributed evenly among maternal BMI classes. There are several possible explanations for this difference. First, it could be explained if the BMI distribution in our sample is significantly different from the general population of pregant women, which would give new defnition to the expected distribution of birth defects in each category if there were to be no difference from the general population. It is also possible that women are underreporting birth defects in children or dropping out of the study prior to data collection due to health concerns for their children. Third, this finding could be representing an underlying confounding factor that is modifying the risk for congenital birth defects in this population overall. Finally, this result could

be an issue of statistical power. We observed a total of 19 children with birth defects; samples that evaluate rates of birth defects often have much larger sample sizes to evaluate.

Further, it is difficult to analyze the distribution of women by BMI category compared to the general population. There is little literature identifying weight distribution of pregnant women in the general population. Future studies that are designed to analyze maternal BMI or weight as a risk factor should consider collecting prepregnancy weight for women to accurately assess pregnancy weight gain and/or prepregnancy BMI. Women in this study vary from 4-33 weeks gestation at their initial study visit, where BMI is assessed. While pregnancy BMI and weight gain can be estimated from this cross-section, there is considerable margin for error in categorizing women based on pregnancy BMI when we do not have a way to obtain a measurement from every participant at the same time point – either prepregnancy or at the same gestational age. The results found in this study are representatiave of the large sample of women in the study, but further study is warranted to determine whether these results are generalizable to women and children throughout rural Appalachia.

In the Pregnancy, Infection, and Nutrition (PIN) Study at the Univesity of North Carolina Hospitals (UNC), women were recruited to study risk factors for preterm birth. This study identified that women who gained more than the recommended amount of weight were at increased risk for Cesarean delivery, high birth weight, macrosomia, and postpartum weight retention (Ferrari & Siega-Riz, 2013). This study explored pregnancy weight gain in three different ways: (1) as a continuous variable of total pregnancy weight gain in kg, (2) as a ratio of observed to expected weigh gain, calculated by dividing the total weight gain in kg by the expected weight gain for gestational age, and (3) as a categorical variable of inadequate, adequate, or excessive weight gain (Ferrari & Siega-Riz, 2013). Implementation of these methods to study pregnancy

weight gain would strengthen the maternal weight variable in our study. Many studies acknowledge that prepregnancy measurements are not feasible and that there is reasonable accuracy of a self-reported prepregnancy weight (Brunner Huber, 2007; Ferrari & Siega-Riz, 2013).

Two interesting findings from our study are the differences in time to first tooth eruption. Children from West Virginia had first tooth eruption significantly earlier than Pittsburgh children, and macrosomic children had first tooth eruption significantly earlier than normosomic children. We have already stated that there were significantly more obese mothers from West Virginia than from Pittsburgh. This result comes in light of the finding that there was no significant difference in the rate of macrosomia between the two sites. In concordance with the previously cited literature (Declerck et al., 2007; Tyrrell et al., 2016; C. Un Lam et al., 2017, 2017), we would expect that differences in macrosomia and differences in tooth eruption would be seen in the same study sample. This necessitates further investigation into potential differences in risk factors for women and children in West Virginia that are not captured by this study.

Strengths of this study include the size of the selected sample. Women largely self-selected into the COHRA2 study based on the perception that they were generally healthy people. In this way, we benefitted from obtaining a sample of women who consider themselves healthy from which to assess risk factors. The COHRA2 study is longitudinal and information was available about all study participants through at least the timepoint of a child's third birthday. Our study is able to assess risk factors that may impact individuals over time, versus assessing prevalence data from rural Appalachia at one point in time.

The study's main pitfall was that prepregnancy weights were not available for the women in the study. Because COHRA studies enroll pregnant women and focus on childhood dental

health, prepregnancy weight is not a data point that is collected for women at their first visit. Future studies would benefit from obtaining this data point for comparisons in order to assess whether there were any significant differences in the risk associated with prepregnancy overweight/obesity and increased pregnancy weight gain. The IOM advises that many studies of maternal determinants of pregnancy weight gain have used weight at the first visit to calculate BMI if the visit occurs prior to 16 weeks gestation, or by recalled prepregnancy weight (Institute of Medicine (US) Committee on Nutritional Status During Pregnancy and Lactation, 1990). Future studies of this nature from COHRA2 participant data should use this gestational timeline as a parameter for defining the study sample. Alternatively, a logarithmic equation developed by the Argentinian Ministry of Health (see Appendix D) has been shown to effectively adjust maternal BMI for gestational age, though still requiring a prepregnancy weight for the function to categorize women by pregnancy BMI (Davies et al., 2013; Ministry of Health and Environment of the Nation, National Directorate of Maternal and Infant Health, 2017).

Additionally, there is a potential that we have underrepresented congenital birth defects in our study. Data were available for participants who at least completed their third study visit, when babies are 10 weeks old. Individuals who had more pregnancy complications or more severe birth defects may not have completed any follow-up study visits and therefore were not included in our data set.

These results necessitate further study on risk factors for pregnancies in northern Appalachia. Notable differences were present in Pittsburgh and West Virginia, which warrant further investigation. We have identified important factors which may be determinants of childhood health outcomes beginning earlier than previously considered for this population. A cohort study of U.S. women and children identified that maternal weight gain during pregnancy

was positively associated with prevalence of overweight children at 3 years of age (Li et al., 2019; Oken et al., 2009; Stamnes Køpp et al., 2012), which draws importance to our finding that 20% of macrosomic children were at or above the 95th percentile for growth at age three. Additionally, Li et al. found that after adjusting for sex of the infant, maternal age, gestational weeks at birth, and gestational weeks when maternal weight was measured, there was still a positive association between both maternal pre-pregnancy BMI and weekly gestational weight gain with physical growth in the child (Li et al., 2019).

The COHRA studies also enroll African American women into the study. African American women experience different health risks during pregnancy (ACOG, 2019). Additionally, African American women have the highest rate of obesity (57%) of all women in the U.S. and have a higher rate of weight retention and obesity after delivery compared to Cauasian women (Hales, 2017; Meng et al., 2018; Walker et al., 2011). Lower rates of common birth defects are reported in African American children (Canfield et al., 2014). It is of interest to determine any differences or similarities observed when considering the two ethnic groups in the COHRA2 study sample, which may further define differences we have observed between West Virginia and Pittsburgh study subsets or identify important differences in health status.

Another important finding that necessitates further investigation is the higher prevalence of diabetes in our study sample. We also saw an increase in Cesarean deliveries in West Virginia. Reasons for this difference should be investigated to determine where and how to prevent adverse maternal and childhood health outcomes associated with Cesarean delivery.

3.5 Conclusions

Overall, these findings bring imperative consideration to obesity as a disease phenotype. We see that even in the absence of other risk factors (i.e., diabetes), obesity is associated with increased rates of Cesarean deliveries and macrosomic infants. Identifying that obesity is a risk factor is an important consideration for how to care for pregnant women. Our findings illustrate that even when no other risk factors have been identified, children may have increased odds of being macrosomic and/or obese in childhood, or subject to earlier tooth eruption and subsequent childhood caries as a result of having an obese mother.

The COHRA2 study sample also illustrates demographic differences that healthcare providers should consider. For example, we saw significant differences in the presence of risk factors and adverse health outcomes such that West Virginia women were more likely to be obese and have Cesarean deliveries. Additionally, children in West Virginia had an earlier time to first tooth eruption. The implications of these significant differences in risk factors for further health problems may be helpful in designing effective public health interventions for this population.

4.0 Relevance to Genetic Counseling and Public Health

Several important public health issues interact to increase the importance of investigating the current issues. The worldwide rate of overweight or obesity in adult women has increased from 29.8% to 38% between 1980 and 2013 (Rahman et al., 2015). Early childhood caries (ECC) has been reported to be the most common chronic disease in United States children. The data gathered during the COHRA2 study were uniquely situated to observe prevalence of risk factors in this population, and while the overarching study initiatives aim to identify factors contributing to oral health, we were able to identify some potential risk factors for early childhood health that begin in the prenatal setting.

Geographic area is a contributing factor to both rising rates of obesity and primary tooth eruption. Patterns of overweight and obesity differ between countries, regions, and income, such that obesity is more prevalent among men in developed countries and among women in developing countries (Ng et al., 2014; Rahman et al., 2015). In 2013, the United States was home to the most obese individuals, accounting for 13% of the 693 million obese individuals worldwide (Ng et al., 2014).

In 2015, a meta-analysis of studies on risk factors related to maternal BMI in mothers from developing countries developed population-attributable risk (PAR) scores to evaluate the effect of geographical region/ sociocultural background on adverse pregnancy outcomes. This study found that overall, developing countries have a disease burden that is double that of developed countries for perinatal and maternal health outcomes that are attributable to maternal BMI (Rahman et al., 2015). Figure 9 shows the PAR scores that the study found to be the proportion of adverse outcomes related to maternal BMI by country.

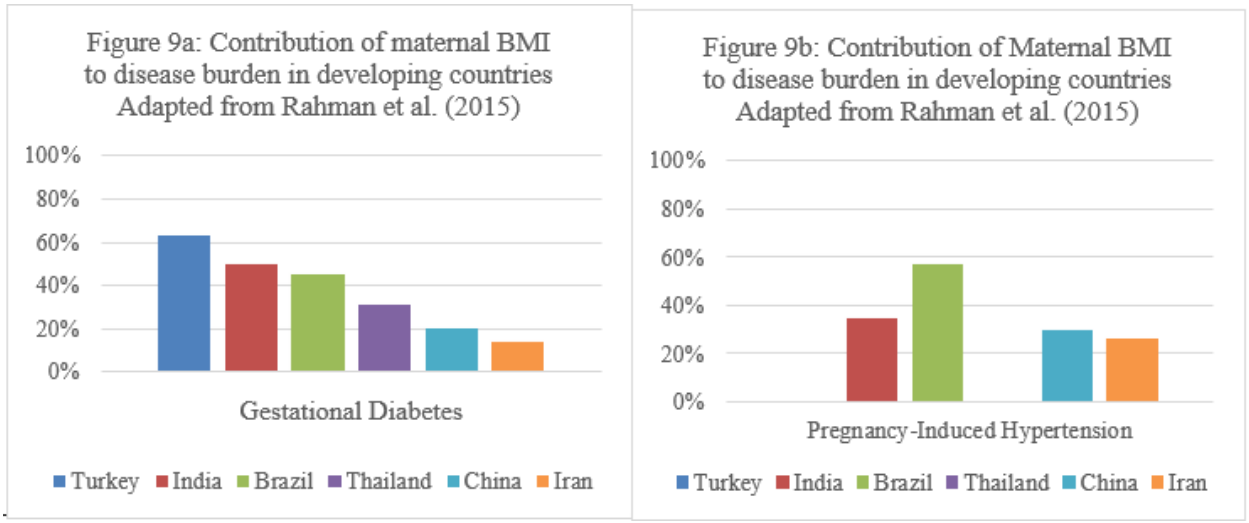


Figure 9: Maternal BMI Associations with Disease Burden

Rahman et al. also found an increased risk for preeclampsia, cesarean delivery, and post-partum hemorrhage in women in overweight and obese categories compared with women in the normal BMI category (Rahman et al., 2015). Further, these authors support that women in these lower-income countries should be counseled about optimal BMI prior to conception due to the lack of nutritional intervention and/or health care system support for interventions to prevent adverse outcomes from pregnancy (Rahman et al., 2015). Additionally, distinct geographical patterns mark areas of increased child and adolescent obesity worldwide. Higher rates of child and adolescent obesity are seen in Middle-Eastern countries, North Africa (primarily for girls), Pacific Islands, and Caribbean nations (Ng et al., 2014).

Our findings suggest that women in northern Appalachia face similar risks for unhealthy BMI as women in other regions around the world. Further, the children in our study – especially those in West Virginia – show signs of developing adverse health outcomes in relation to maternal BMI. Pregnant women in this region would benefit from guidelines that support healthy weight gain during pregnancy based on pre-pregnancy BMI. The Maternal Obesity and Childhood

Outcomes (MOCO) consortium identified 50 European, North American, and Oceanian cohorts to develop international gestational weight gain charts for specific pre-pregnancy BMI categories, which will improve the risk assessment and risk reduction for pregnant women worldwide. Figure 10 shows the MOCO 2018 guideline for healthy gestational weight (Santos et al., 2018).

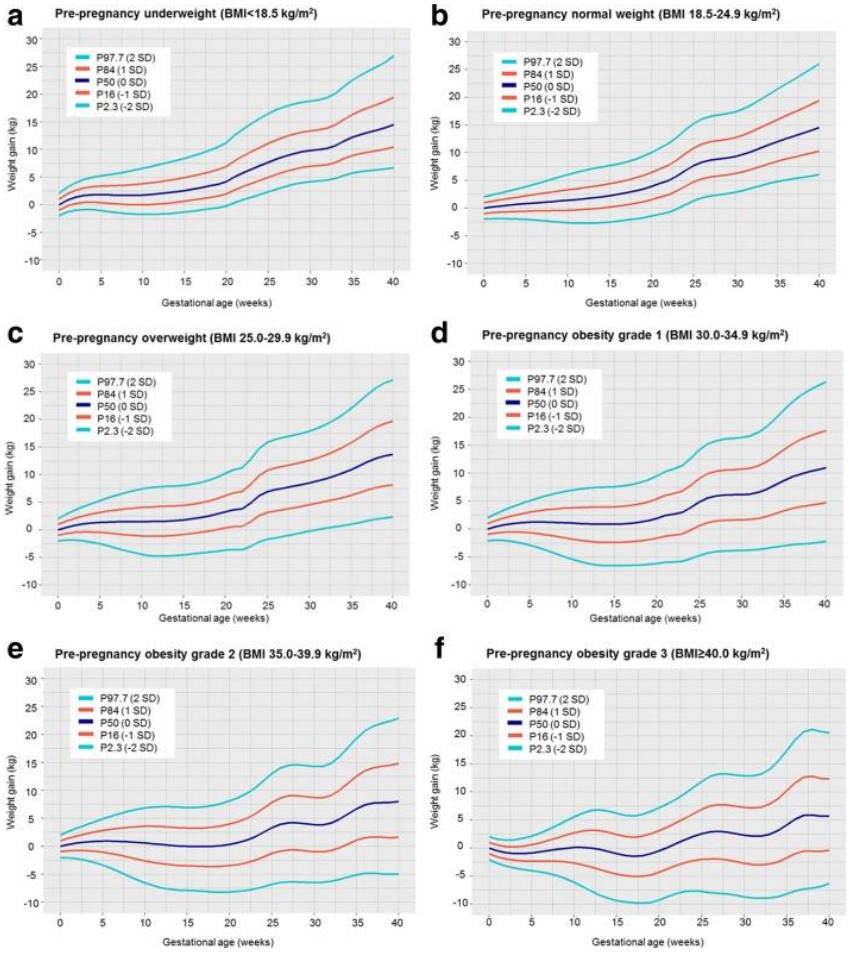


Figure 10 Maternal Obesity and Childhood Outcomes (MOCO)

Selected percentiles of weight gain for gestational age in women without any pregnancy complication for maternal pre-pregnancy underweight(a), normal weight(b), overweight (c), obesity grade 1(d), obesity grade 2(e), and obesity grade 3(F)

Evidence across generations and studies suggests that geographic region marks differences in the eruption timing of children's primary teeth (Carolina Un Lam et al., 2016). Even within the United States, the timing of tooth eruption varies between American Indian, Black, and White children (Warren et al., 2016). Other ethnic groups – such as northern plains American Indians (Warren et al., 2016) – may experience a significantly different mean age of primary tooth eruption. Individuals of Asian ethnicity have been shown to have significantly delayed tooth eruption when compared to white children (Ntani et al., 2015).

4.1 Socioeconomics

Socioeconomic factors are also important to consider. Previous data show that prevalence of obesity is higher in rural areas than in urban areas (Chen et al., 2018). Our data are consistent with this theory, as demonstrated by the disproportionate rate of obesity in West Virginia. West Virginia, as we have previously stated, represents a region of the United States that is largely rural (it is the only state that is entirely located in Appalachia) and public health initiatives in the state largely focus on increasing access to health services.

As shown in one study, children of mothers on welfare programs and/or living in more deprived areas are more likely to have advanced dental development (Ntani et al., 2015). Children of taller and heavier mothers and mothers who had a poorer quality diet had earlier first tooth eruption, more teeth at one year of age, and were more likely to have advanced dental development, defined as more than 16 teeth at two years of age (Ntani et al., 2015). These findings are consistent with the idea that individuals in lower socio-economic standing eat lower-cost foods which tend to have an increased sugar content, increasing the risk of dental caries development (Julihn et al.,

2009). Conversely, a study by Un Lam et al. found that intake of high-sugar foods was unrelated to early childhood caries at two years of age (C. Un Lam et al., 2017). The same study found that Indian mothers consumed more cheese and yogurt – and therefore presumably had a higher calcium intake – than Chinese and Malay mothers in the study; additionally, the children in the study with Indian mothers experienced a lower caries rate than the other two ethnic groups (C. Un Lam et al., 2017). They hypothesize that early childhood caries in toddlers are more associated with biological factors, and that plaque deposition is more affected by environmental or behavioral exposures in two-year-olds (C. Un Lam et al., 2017).

4.2 Women Referred for Genetic Counseling

We have shown that maternal weight is an important factor in assessing what will be a risky pregnancy. Genetic counselors are specifically trained to support patients in obtaining information and resources that will promote health and well-being for families. It is within reason for a genetic counselor to anticipate and assuage feelings of uncertainty or guilt surrounding risk information for patients. The findings from our study may be useful to genetic counselors in advocating for patients by providing accurate risk in formation regarding BMI during pregnacy.

Establishing the relationship between common (complex) disease phenotypes such as obesity or diabetes is important for future genetics studies in order to develop better clinical practices. Genome-wide association studies (GWAS) are a helpful tool for researchers to investigate the contribution of novel genes to complex disease phenotypes. Obesity is a complex disease phenotype with many factors, and diabetes mellitus is often a comorbidity of obesity. Both are known to have genetic components, and we have previously stated that both are known risk

factors during pregnancy. It was previously known that there are multiple genes associated with type II diabetes. Recent studies have shown that several genes known to be associated with type II diabetes are also associated with gestational diabetes. Table 5 is adapted from Lowe Jr. and Karban (2014) and describes eight genetic loci known to be associated with both type II diabetes and gestational diabetes (Lowe Jr & Karban, 2014).

Table 5: Genes Associated with Gestational Diabetes

Genes with known associations with type II diabetes that demonstrate association with gestational diabetes.		
Gene	**Protein**	**Function**
IRS1	Insulin receptor substrate 1	Substrate of insulin receptor tyrosine kinase
IGF2BP2	Insulin-like growth factor 2 mRNA-binding protein 2	Binds insulin-like growth factor 2 mRNA and may regulate protein translation; risk allele associated with decreased insulin secretion
CDKAL1	CDK5 regulatory subunit associated protein 1 like-1	Unknown function
GCK	Glucokinase	Phosphorylates glucose in pancreatic B-cells and hepatocytes and involved in the regulation of insulin secretion.
TCF7L2	Transcription factor 7-like 2	Transcription factor and member of the Wnt signaling pathway. Risk allele associated with reduced insulin secretion.
MTNR1B	Melatonin receptor 1B	G-protein coupled receptor that is expressed on B-cells, binds melatonin, and may antagonize insulin release
KCNJ11	Potassium inwardly rectifying channel, subfamily J, member 11	Integral membrane protein and inward-rectifier type potassium channel that is controlled by G-proteins and associated with sulphonyl urea receptor; involved in regulation of insulin secretion
KCNQ1	Potassium voltage-gated channel, KQT-like subfamily, member 1	Voltage-gated potassium channel; involved in the regulation of insulin secretion

Obesity risk genes that have been found to be involved in gestational weight gain include *FTO, MC4R, TMEM18,* and *KCTD15* (Groth & Morrison-Beedy, 2015; Lawlor et al., 2011; Martins et al., 2016; Meng et al., 2018; Stuebe et al., 2010). As more is understood about the role of genes in weight gain, it will be important to consider how this information becomes increasingly

relevant to clinicians who care for pregnant women who rapidly gain weight in a short amount of time.

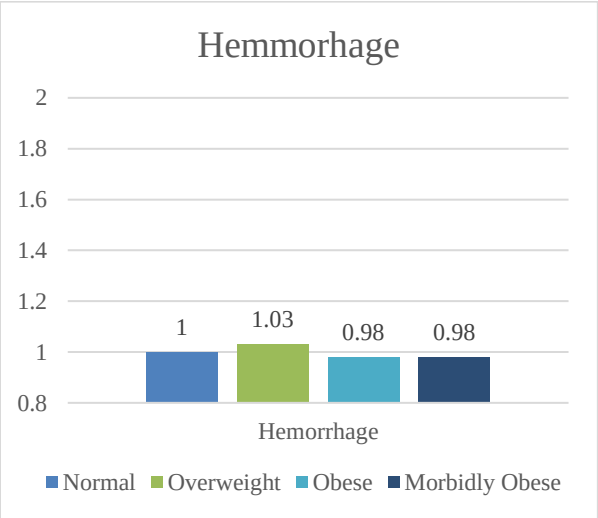
In the Pregnancy and Childhood Epigenetics (PACE) Consortium, Sharp et al. showed that BMI at the start of pregnancy was associated with differential methylation in newborn blood, such that children displayed epigenetic patterns associated with a higher risk of obesity and obesity-related disorders later in life (Sharp et al., 2017).

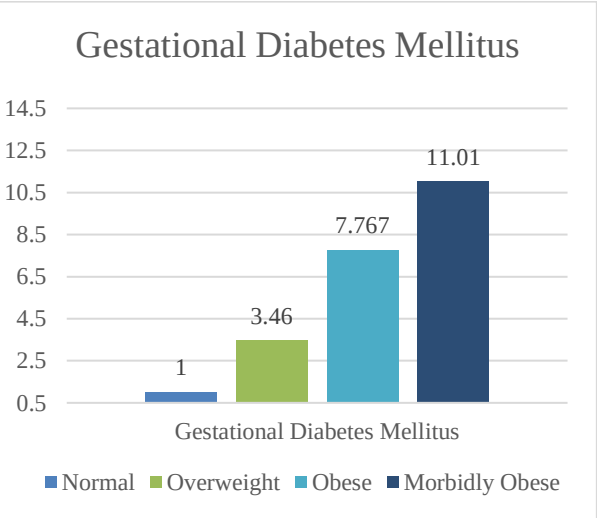
Considering the genetic components of these disease phenotypes will become increasingly important as we consider new genetic technologies and how they will impact patients. While our study suggests that complex disease phenotypes may have a negative impact on a developing fetus, research on the genetics of these diseases may uncover new ways in which patients would benefit from genetic testing. In the future, genetic counselors may be called to discuss how knowing a patient's genetic risk of developing gestational diabetes could be beneficial to managing their pregnancy.

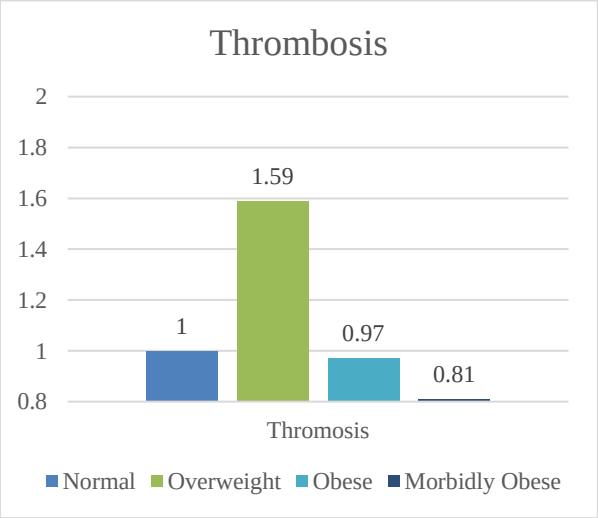
Research shows that women who reported being advised about a healthy weight gain were more likely to gain within the recommended range than women who do not receive such guidance (Ferrari & Siega-Riz, 2013; Goswell et al., 1999; Stotland et al., 2005; S M Taffel & Keppel, 1986; Selma M. Taffel et al., 1993). In the UNC Hospitals PIN Study, 51.8% of women reported receiving weight gain advice by 27-30 weeks of pregnancy; there were no significant associations with whether or not women received advice based on race, age, marital stuatus, or prepregnancy BMI. Ninety-one percent of these women reported following the advice, however, when controlling for hypothesized confounders and mediators, there was no evidence for an association between provider advice and pregnancy weight gain, and two thirds of women gained more than the IOM recommended weight during pregnancy (Ferrari & Siega-Riz, 2013).

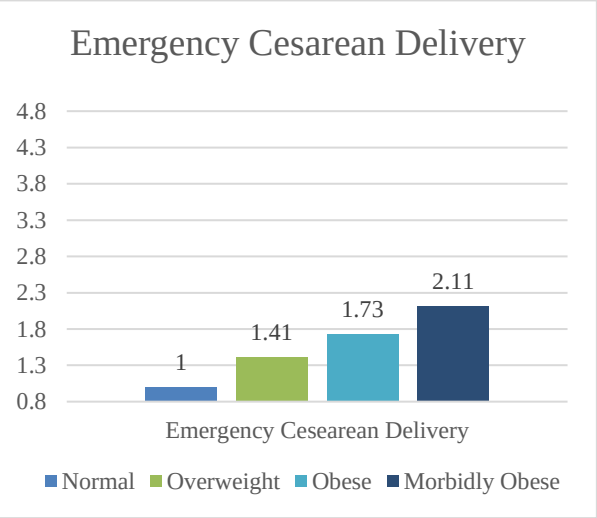
Women who will be 35 years or older at delivery should be referred to a genetic counselor to discuss the increased risks for chromosomal aneuploidies in this population. Wu et al. (2019) found a significantly earlier tooth eruption for children born to mothers over the age of 35 (Wu et al., 2019). Previous findings show a relationship between maternal age and child growth parameters such that an increase in childbearng age was associated with taller stature in prepubescent children (Savage et al., 2013) Similarly, other studies show that maternal age (>30 years) is a risk factor for fetal macrosomia (Usta et al., 2017). Sessions with genetic counselors in this population may elicit risk factors for later health implications in children. As these relationships become better understood, it is worthwhile to provide genetic counselors with educational materials for their patients regarding the risks associated with increased weight during pregnancy. Further, knowledge of these risk factors may help genetic counselors to counsel their patients in the presence of a negative test result for a genetic etiology for a known birth defect.

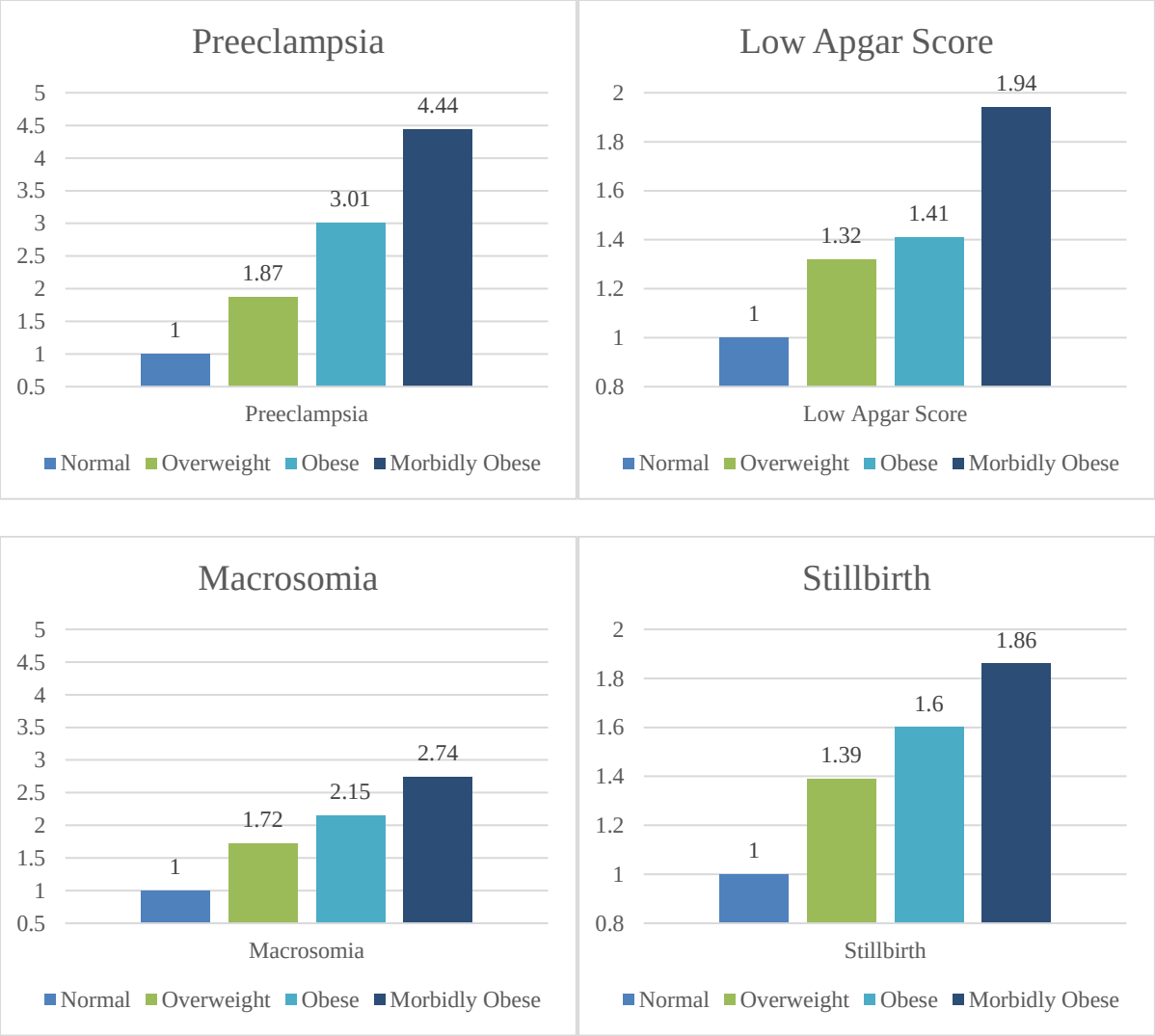
Appendix A Increased Maternal BMI and Adverse Pregnancy Outcomes











Appendix Figure 1: Adverse Pregnancy Outcomes Associated with Pregnancy BMI

Figures are adaptations of results from Ovesen et al. 2011.

Ovesen et al. found that there were significant differences in the risks for multiple adverse health outcomes if mothers were overweight or obese. The figures above illustrate the odds ratios found in their study. As these results show, there are increased odds of both adverse maternal outcomes and fetal outcomes. Gestational diabetes mellitus (B), emergency cesarean delivery (D), preeclampsia (E), low Apgar scores (F), macrosomia (G), and stillbirth (H) all showed dose

dependence, such that more obese women had higher odds of having adverse health outcomes compared with normal weight women (Ovesen et al., 2011).

Appendix B Obesity in Children That Were Macrosomic At Birth

Girls			Boys		
Age 1 Weight	Age 2 Weight	Age 3 Weight	Age 1 Weight	Age 2 Weight	Age 3 Weight
8.63	11.3	13	8.95	11.4	12.7
10.19	13.4	14.5	-9999	15.9	-9999
9.979024	15.7	18.18	12.62	15.1	17.2
10.20582	14.06	16.8	9.07	12.17	13.55
9.525432	15.79	-9999	8.18	11.35	-9999
10.23	-9999	-9999	10.75	13.2	15.2
9.62	11.81	14.4	11.36	14.3	16.51
9.01	12.6	14.35	11.158	14.35	16
11.2	15.5	17.3	10.3	12.3	14.4
8.797	12.246	14.15	9.6	11.9	14.1
10.945	13.18	13.27	10.1	13.6	13.63
-9999	12.7	15	10.1	13.3	15.05
8.93	11.8	14.6	10.95	12.55	15.05
-9999	13.2	16.1	9.9	13.3	15.2
9.72	13.1	15.5	11.32	14.1	-9999
10.09	11.75	13.7	10.94	14.3	16.6
11.47	14.5	16.2	9.84	11.8	12.9
10.659412	13.063	-9999	9.241937	13.6	-9999
12.2	14.3	-9999	-9999	13.5	17.1
11.15	13.7	20.41	11.18	14.06	-9999
9.6	11.82	14.243	10.42	13.85	15.7
10.82	-9999	-9999	10.432616	-9999	-9999
-9999	12.6	15	-9999	13.64	17.64
-9999	14.061	-9999	9.98	12.4	13.61
11.3	12.6	-9999	10.251	12.73	15.54
-9999	15.9	19.8	11.21	13.38	-9999
9.97	13	14.6	9.525432	14.9	-9999
11.14	-9999	-9999	9.25	11.85	14
11.5	14.6	-9999	10.35	14.3	16.7
11.7	15.33	19.3	11.5382465	14.2	-9999
10.659	12.5	12.85	8.3064035	11.33	13.2
			10.38	13.33	-9999
			11.9	13.608	15.97
			9.8	13.1	14.9
			9.5537815	-9999	15.5
			-9999	14.6	-9999
			10.5	15.02	14.45
			11.39	14	-9999
			12.700576	15.15	17.9
			10.41	12.37	13.88
			12.61	16.67	16.45
			10.08	12.3	14.9
			11	13.7	17.14
			10.67	13.7	16.4
			12	16.05	18.9
			-9999	12.5	15.45
			12.32	15.9	17.4
			11.5	17.3	-9999
			10.97	15	18.2
			13.1258185	15	-9999
			10.45	-9999	-9999
			11.37	13.8	15.9
			10.9145575	15.331	-9999
			11.32	-9999	17.4
			-9999	-9999	16.36
			9.18	13.24	17.4
			11.3398	15.241	-9999
			10.57	12.6	16.4
			10.66	16.2	16.4
			-9999	12.9	15.2
			10.149121	12.45	13.9
			10.0073735	13.9	-9999
			11.7083435	14.8	16.91
			11.226402	14.061	17.82
			12.03	14.3	16

Appendix Figure 2: Weights of Children who were Macrosomic at Birth

Children's weights are shown in kilograms. Using the Centers for Disease Control and Prevention's data tables for childhood length-for-weight, we determined which macrosomic infants in our study were obese (>95th percentile for growth) at their first, second, and third birthdays (in red). These tables are standardized for male and female children. Some children were consistently obese throughout early childhood. Others normalized as they aged, and some alternated between overweight and obese. The negative number code (-9999) represents a time point at which data were not collected, most likely where the participant had discontinued from the study.

Appendix C Congenital Birth Defects Reported in COHRA2 Infants

Appendix Table 1: Congenital Birth Defects Reported in COHRA2 Infants

Congential Defect Reported	Delivery Method	Child's Weight Category	Mother's BMI Category
Heart murmur	Vaginal	Normosomic	Overweight
Heart murmur and spina bifida	Cesarean	Normosomic	Overweight
Heart murmur	Cesarean	Normosomic	Obese
Heart murmur and left-sided deafness	Cesarean	Normosomic	Normal weight
Heart murmur	Cesarean	Normosomic	Overweight
Heart murmur	Vaginal	Normosomic	Nomal weight
Heart murmur	Vaginal	Normosomic	Normal weight
Polydactyly and syndactyly	Vaginal	Normosomic	Overweight
Craniosynostosis	Cesarean	Normosomic	Obese
Ventral septal defect	Vaginal	Normosomic	Overweight
Heart murmur	Vaginal	Normosomic	Overweight
Patent ductus arteriosis	Vaginal	Normosomic	Obese
Heart murmur	Vaginal	Normosomic	Overweight
Heart murmur	Vaginal	Normosomic	Normal weight
Heart murmur	Cesarean	Normosomic	Overweight
Tetgralogy of fallot	Vaginal	Macrosomic	Normal weight
Heart murmur	Cesarean	Macrosomic	Normal weight
Craniosynostosis	Vaginal	Macrosomic	Obese
Polydactyly	Vaginal	Macrosomic	Overweight

This table lists the collective congenital birth defects reported in COHRA2 child participants that were determined to be isolated (not due to a genetic or syndromic condition). Additionally, information on each child's mother's body mass index, delivery method, and weight classification are shown. No significant relationships were identified in this study subset. Nineteen children were reported as having an isolated birth defect, representing 1.9% of the study sample. Further information on inclusion criteria for continuation in the COHRA2 study is needed to

determine whether this percentage reflects the total number of children who were reported to have a birth defect. As such, the rate of birth defects in this study sample is not different from the general population (2-3%) and n is not large enough for any further comparisons.

Appendix D Equation for Determining Gestational BMI

The following equation was developed by the Argentinian Ministry of Health to adjust maternal BMI for gestational age (GBMI = gestational BMI).

If pregravid BMI is ≥ 21 to < 25 kg/m^2, then the equation [(weight-5.5) / (height^2)] = GBMI. GBMI can be categorized as follows:

Appendix Table 2 Ministry of Health Gestational BMI Categories

GBMI	**Category**
≥10 to ≤ 19.8 kg/m^2	Underweight
≥19.8 to ≤ 26.1 kg/m^2	Normal Weight
≥26.1 to ≤ 29 kg/m^2	Overweight
≥29 to ≤ 50 kg/m^2	Obese

(Davies et al., 2013; Ministry of Health and Environment of the Nation, National Directorate of Maternal and Infant Health, 2017).

Appendix E IRB Approval Forms

University of Pittsburgh
Institutional Review Board

Human Research Protection Office
3500 Fifth Avenue, Suite 106
Pittsburgh, PA 15213
Tel (412) 383-1480
www.hrpo.pitt.edu

APPROVAL OF SUBMISSION (Expedited)

Date:	September 27, 2019
IRB:	STUDY19080178
PI:	Mary Marazita
Title:	COHRA2: Factors Contributing to Oral Health Disparities in Appalachia: Coordinating Center
Funding:	Name: National Institute of Dental and Craniofacial Research , Funding Source ID: DE014899
Grant Title:	None

The Institutional Review Board reviewed and approved the above referenced study. The study may begin as outlined in the University of Pittsburgh approved application and documents.

Approval Documentation

Review type:	Continuing Review
Approval Date:	9/27/2019
Expiration Date:	9/26/2020

Determinations:	None
Approved Documents:	• COHRA2 CC Consent Template Adult 10-19-11.pdf, Category: Consent Form; • COHRA2 CC Consent Template Child 10-19-11.pdf, Category: Consent Form

As the Principal Investigator, you are responsible for the conduct of the research and to ensure accurate documentation, protocol compliance, reporting of possibly study-related adverse events and unanticipated problems involving risk to participants or others. The HRPO Reportable Events policy, Chapter 17, is available at http://www.hrpo.pitt.edu/.

Continuing review (CR) can be submitted by clicking "Create Modification/CR" from the active study at least 5 weeks prior to the expiration date.

Clinical research being conducted in an UPMC facility cannot begin until fiscal approval is received from the UPMC Office of Sponsored Programs and Research Support (OSPARS).

If you have any questions, please contact the University of Pittsburgh IRB Coordinator, Dana DiVirgilio.

Please take a moment to complete our Satisfaction Survey as we appreciate your feedback.

University of Pittsburgh
Institutional Review Board

Human Research Protection Office
3500 Fifth Avenue, Suite 106
Pittsburgh, PA 15213
Tel (412) 383-1480
www.hrpo.pitt.edu

APPROVAL OF SUBMISSION (Expedited)

Date:	November 18, 2019
IRB:	STUDY19110013
PI:	Mary Marazita
Title:	COHRA2: Factors Contributing to Oral Health Disparities in Appalachia: Pennsylvania Sites
Funding:	Name: National Institute of Dental and Craniofacial Research , Funding Source ID: DE014899; Name: National Institute of Dental and Craniofacial Research , Funding Source ID: DE014899
Grant Title:	None

The Institutional Review Board reviewed and approved the above referenced study. The study may begin as outlined in the University of Pittsburgh approved application and documents.

Approval Documentation

Review type:	Initial Study
Approval Date:	11/18/2019
Expiration Date:	11/26/2020

Determinations:	None
Approved Documents:	• COHRA2 Consent Form Adult Ages 7 - 10 12-10-18.pdf, Category: Consent Form; • COHRA2 Consent Addendum Adult 8-31-16.pdf, Category: Consent Form; • COHRA2 Consent Addendum Child 8-31-16.pdf, Category: Consent Form; • COHRA2 Consent Form Adult 9-6-18.pdf, Category: Consent Form; • COHRA2 Consent Form Adult Ages 2 - 6 9-6-18.pdf, Category: Consent Form; • COHRA2 Consent Form Child 9-6-18.pdf, Category: Consent Form; • COHRA2 Consent Form Child Ages 2 - 6 1-9-18.pdf, Category: Consent Form; • COHRA2 Consent Form Child Ages 7 - 10 12-10-18.pdf, Category: Consent Form;

As the Principal Investigator, you are responsible for the conduct of the research and to ensure accurate documentation, protocol compliance, reporting of possibly study-related adverse events and unanticipated problems involving risk to participants or others. The HRPO Reportable Events policy, Chapter 17, is available at http://www.hrpo.pitt.edu/.

Continuing review (CR) can be submitted by clicking "Create Modification/CR" from the active study at least 5 weeks prior to the expiration date.

Clinical research being conducted in an UPMC facility cannot begin until fiscal approval is received from the UPMC Office of Sponsored Programs and Research Support (OSPARS).

If you have any questions, please contact the University of Pittsburgh IRB Coordinator, Dana DiVirgilio.

Please take a moment to complete our Satisfaction Survey as we appreciate your feedback.

West Virginia University
Office of Research Integrity and Compliance
886 Chestnut Ridge Road Morgantown, WV 26506

Approval of Protocol Renewal

10/09/2019

To: Daniel McNeil

From: WVU Human Research Protection Program

Protocol Type: Expedited

Approval Date: 10/09/2019

Submission Type: Renewal

Expiration Date: 10/08/2021

Funding: University of Pittsburgh

WVU Protocol #: 1411480509R006

Protocol Title: Factors Contributing to Oral Health Disparities in Appalachia

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The West Virginia University Institutional Review Board has reviewed and granted your request for re-approval of Expedited protocol 1411480509R006, in accordance with the Federal regulations 45 CFR 46, 21 CFR 50, and 21 CFR 56 (when applicable). Additional details concerning the review are below:

- Category 3. This research study was granted an exemption because this (i) Research involves Benign Behavioral Interventions through verbal, written responses, (including data entry or audiovisual recording) from adult subject who prospectively agrees and ONE of following met: A. Recorded information cannot readily identify the subject (directly or indirectly/linked) B. Any disclosure of responses outside of the research would NOT reasonably place subject at risk (criminal, civil liability, financial, employability, educational advancement, reputation) C. Information is recorded with identifiers & IRB conducts Limited Review 104(d)(3)(i)
- Category 7. Research on individual or group characteristics or behavior (including, but not limited to, research on perception, cognition, motivation, identity, language, communication, cultural beliefs or practices, and social behavior) or research employing survey, interview, oral history, focus group, program evaluation, human factors evaluation, or quality assurance methodologies. [NOTE: Some research in this category may be exempt from the DHHS regulations for the protection of human subjects. See Exempt Categories and 45 CFR 46.101(b)(2) and (b)(3). This listing refers only to research that is not exempt.]

The following documents were reviewed and approved for use as part of this submission. Only the documents listed below may be used in the research. Please access and print the files in the Notes & Attachments section of your approved protocol.

- List of Personnel using De-Identified Data - COHRA 2 - Jul 7 2019.pdf
- COHRA2 Procedures Manual v2.3 1-10-19.docx
- CROMS Protocol v1.9 19-1-10.docx
- Consent - Jul 22 2019.pdf
- Assent - Jun 7 2019.pdf
- Ripple Ads.pdf
- -WVU - Dental Research 30 ifile.mp4
- Tissue Banking Form.pdf
- Mental Health Resources 10-10-17.pdf
- COHRA2 Perceived Discrimination v1.pdf
- Participant Satisfaction Survey.pdf
- McNeil approval letter.pdf
- Children's Medication Inventory v1.0 - SNAP Questionnaire for IRB 4-27-16.pdf
- COHRA SMILE - Tri-fold - ad brochure.pdf
- COHRA SMILE ad flyer - tear off.pdf
- ASRS-5 - Adult ADHD Self-Report Screening Scale.pdf
- Facility List - Jul 22 2019.pdf
- CBCL - Ages 6 - 18.pdf
- CBCL, Consent to Contact, Discontinuation.pdf
- ID Form & Consent Flowsheet.pdf
- Eligibility, Contact & Water Forms.pdf
- Prenatal Med, Dental Fear & Anxiety & F2F Prenatal.pdf
- Ripple Security.pdf
- Ripple HIPAA.pdf
- Occlusion Questionnaire 3-21-18.pdf
- Radio ad - Revised - Apr 10 2018.pdf
- Med Inv Postnatal, F2F Birth Outcome, F2F Postnatal.pdf
- Adult & Child Dental Assessments.pdf
- Bundle.pdf
- CFSS - Dental Subscale.pdf
- COHRA2 Prenatal Phone Questionnaire.pdf
- Ruggles (Johnson) - CITI.pdf
- COHRA2 Postnatal Phone Questionnaire v2.12 7-19-17 final to UCSUR.pdf
- COHRA2 Postnatal Phone Questionnaire 2.5 years v1.0 7-19-17 final to UCSUR.pdf
- COHRA2 Postnatal Phone Questionnaire v2.12 7-19-17 final to UCSUR.pdf
- mcneil qip report.docx
- COHRA2 Postnatal Phone Questionnaire 2.5 years v1.0 7-19-17 final to UCSUR.pdf

WVU IRB approval of protocol 1411480509R006 will expire on 10/08/2021.

Once you begin your human subjects research, the following regulations apply:

1. Unanticipated, serious adverse events and/or side effect(s) encountered at WVU or an affiliate site that are related to the research must be reported to the WVU IRB within five (5) days using the Notify IRB action in WVU+kc.
2. Any Unanticipated Problem or UPIRTSO or other research related event resulting in new or increased risk of harm to study subjects, occurring at WVU or an affiliate site, must be reported to the WVU IRB within five (5) days using the Notify IRB action in WVU+kc.
3. Any modifications to the protocol or informed consent form must be reviewed and approved by the IRB prior to implementation. These modifications should be submitted as an amendment.
4. You may not use a modified informed consent form until it has been reviewed and approved by the WVU IRB. **Only consent forms with the WVU+kc watermark may be used to obtain informed consent from participants.**

The WVU Human Research Protection Program will be glad to provide assistance to you throughout the research process. Please feel free to contact us by phone at 304.293.7073 or by email at IRB@mail.wvu.edu.

Sincerely,

Lilo Ast
IRB Administrator

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