PREECLAMPSIA AND FETAL GROWTH: INFLUENCE OF INFANT SEX

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In response to in utero insults, male vs. female infants have greater disadvantages in pregnancy outcome. We asked if this differential impact of fetal sex might extend to fetal growth in utero during preeclampsia. We first investigated the influence of relevant variables in normotensive pregnancy. We evaluated whether the impact of maternal pre-pregnancy body mass index (BMI), smoking and socioeconomic status were modified by sex and/or race in singleton offspring of 8,801 primiparous normotensive women enrolled in the Collaborative Perinatal Project. The mean head-to-chest circumference (HCC) decreased more or each 1kg/m^2 increase in pre-pregnancy BMI, while mean birthweight and ponderal index (PI) increased more for each 1kg/m^2 increase in pre-pregnancy BMI among term females vs. males ($p=0.07$, $p<0.01$ and $p=0.08$, interaction respectively).

We then investigated whether the relationship between preeclampsia and fetal growth was modified by sex in offspring of 516 preeclamptic and 8801 normotensive primiparous women. Male vs. female preterm offspring of preeclamptic mothers had greater reductions in mean birthweight, head and chest circumferences ($p=0.05$, $p=0.02$, $p=0.01$; interaction respectively). The influence of preeclampsia on growth of term offspring was more modest, and the influence of sex was opposite that in preterm infants.

Next we investigated placentas from 735 preeclamptic and 21,185 normotensive primiparous and multiparous women, to determine which dimensions of placental growth are reduced in preeclamptic pregnancies. We then investigated if the relationship between these measures and birthweight was constant between offspring of normotensive and preeclamptic women, as well as across infant sex. We found that the smaller but not the larger placental diameter was an independent predictor of preeclampsia ((smaller diameter <15cm OR 1.27 95% CI 1.01, 1.59) and larger diameter <18 cm (OR 1.18 95% CI 0.90, 1.54)). We also found higher rates of increase in birth weight at lower placental weight and placental diameters in offspring of preeclamptic vs. normotensive women (all $p<0.05$, interaction). Additionally, we found that among the offspring of preeclamptic women, female offspring with smaller diameters above 20cm, had a reduction in birth weight while males did not ($p=0.02$, interaction).

This work yields meaningful public health findings by providing evidence that influences upon fetal and placental growth are different by infant sex. Studies of mechanisms affecting fetal growth should investigate interactions with fetal sex. We hope studies of the involved biological pathways will direct future research to reduce rates of growth restriction and later life chronic diseases.

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1.0 INTRODUCTION

1.1 SPECIFIC AIMS

Fetal growth restriction (FGR) is the failure of fetus to grow to his/her genetic growth potential (1). Fetuses with FGR have an increased likelihood of fetal death, need for assisted ventilation (2), cerebral palsy, as well as later life mortality and morbidity(3-8). Sex differences are often observed in pregnancy outcome. Spontaneous preterm labor (9) and complications of pregnancies (such as fetal distress during labor and non-reassuring fetal heart rate patterns) are more common with pregnancies bearing male fetuses compared to female fetuses (10, 11). Emerging evidence indicates that the frequency, expression and outcome of illnesses in adults is different based on sex (12). For example, males have poorer rates of survival from cardiovascular disease (13) and various cancers (14-16) as well as higher rates of Parkinson's disease (17, 18). Women tend to develop cardiovascular disease later in life compared to men and are more likely than men to die within one year of a heart attack (13). Female smokers are at a significantly greater risk of developing lung cancer than male smokers at similar levels of smoking (19). We hypothesize that this differential risk in disease outcome also exists in utero and at birth.

Preeclampsia is a pregnancy specific disease that can cause fetal growth restriction (20-24) and reduced placental growth(25, 26). Based on evidence of sexual differences in several pregnancy outcomes with different fetal sex, we suspect that fetal growth in preeclampsia is different based on the sex of the infant.

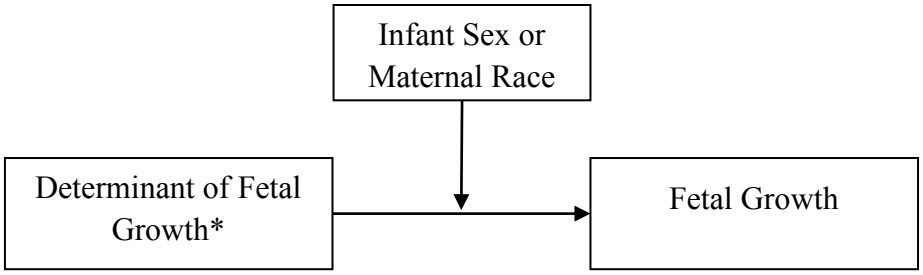
Small for gestational age (SGA) is the most common marker used to detect abnormal fetal growth. It is commonly defined as birthweights below the 10th percentile for babies born at the same gestational age in a population, adjusted for sex and race. However SGA as an indicator of reduced growth has several limitations: 1) An SGA infant may not be growth restricted, e.g. small individuals have small babies. 2) Growth restricted infants who do not exercise their true genetic growth potential may not be small enough to be SGA. Similarly, the ratio of fetal weight to placental weight (FPR) is often used as a measure of the efficiency of fetal growth relative to placental growth (27). However this also has limitations. Placentas that are large and thin as well as placentas that are small and thick can produce FPRs that are consistent with normal proportioned placentas, (27) yet these may have very different implications (28). Owing to these limitations of determining impaired fetal growth by just SGA and FPR, it will be helpful to use additional anthropometric and placental indicators to characterize if fetal and placental growth in pregnancies diagnosed with preeclampsia varies by fetal sex.

Guided by evidence of sexual differences in disease occurrence and severity outside of pregnancy and the known differences in several pregnancy outcomes with different fetal sex **we intend to investigate if the relationship between preeclampsia, fetal growth and placental growth is modified by infant sex.**

To meet the following specific aims, we will use data from the Collaborative Perinatal Project (CPP) — a prospective study of over 58,000 women recruited in 1959 to 1965 that collected obstetrical, perinatal, and pathological data to investigate neurologic disorders in children born to the women in the cohort.

In order to assess the relationship between preeclampsia, fetal growth and fetal sex we first need to better understand the contribution of fetal sex to the markers of fetal growth and determine if sexual and/or racial dimorphisms exist among the determinants of fetal growth.

Specific Aim 1: To determine if maternal pre-pregnancy BMI, smoking and SES contribute differently to fetal growth by infant sex or maternal race.



*Determinant= maternal pre-pregnancy BMI, Smoking or SES.

Figure 1: Specific Aim 1

Hypothesis: Similar to the trend with infant birthweight, these markers of growth: frequency of SGA, mean ponderal index (PI), mean head to chest circumference (HCC), and mean fetal placental ratio (FPR) will be lower for female and Black infants among smokers and lower SES groups. Female vs. male and Black vs. White infants will have a lower rate of growth with increasing maternal pre-pregnancy BMI.

After characterizing the relationship of fetal sex to ponderal index, head-chest circumference ratio and fetal placental ratio in normotensive pregnancies, we will then compare the influence of fetal sex in preeclampsia on the same outcomes. Small-for-gestational age categorization may underestimate the influence of reduced growth; therefore additional markers of growth will also be evaluated.

Specific Aim 2: To investigate if the relationship between preeclampsia and fetal growth is modified by infant sex.

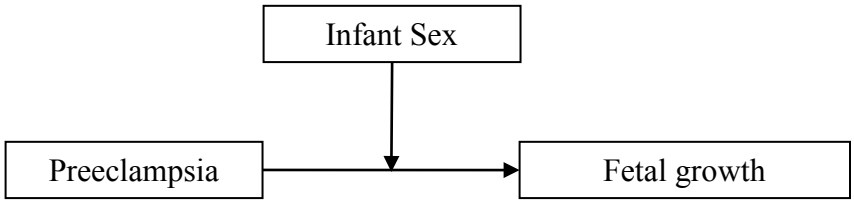


Figure 2: Specific Aim 2

Aim 2a: This study aims to investigate whether the relationship between preeclampsia and fetal growth is different by fetal sex among term infants using these markers of fetal growth: frequency of SGA, mean ponderal index, mean head to chest circumference and mean fetal placental ratio.

Hypothesis: Term infants born to preeclamptic women will have smaller mean ponderal indices, smaller fetal placental ratios and higher head to chest circumference ratios compared to infants born to normotensive women. Reduced growth will be more profound among term male offspring of preeclamptic vs. normotensive women than among term female offspring of preeclamptic vs. normotensive women.

Aim 2b: This study aims to investigate whether the relationship between preeclampsia and fetal growth is different by fetal sex among preterm infants.

Hypothesis: Reduced growth will be more profound among preterm male offspring of preeclamptic vs. normotensive women than among preterm female offspring of preeclamptic vs. normotensive women.

Specific Aim 3a: To investigate which dimensions of placental growth (thickness, small and large diameters and ratio of the diameters) are reduced in association with preeclampsia.

Hypothesis: The risk of preeclampsia will increase with decreasing placental growth in all four placental dimensions.

Specific Aim 3b: To investigate if the relationship between measures of placental growth and birth weight were constant between offspring of normotensive and preeclamptic women.

Specific Aim 3c: To investigate if the relationship between the measures of placental growth and birth weight was constant across male vs. female offspring of normotensive and preeclamptic women.

Hypothesis: In comparison to offspring of normotensive women, the trends in the relationship between birth weight and placental growth (specifically growth of the smaller diameter and larger placental diameter) will be different in preeclamptic pregnancies, as well as by infant sex.

This research intends to bridge the gap in our understanding of fetal growth in normal pregnancy, fetal and placental growth in preeclampsia and the influence of fetal sex on these markers of fetal and placental growth. These relationships may be beneficial in directing future

research to interpret the mechanisms of fetal growth, placental structure/function, the pathophysiology of preeclampsia and the influence of fetal sex on these markers of growth.

1.2 BACKGROUND

1.2.1 Preeclampsia

1.2.1.1 Significance of preeclampsia

Preeclampsia is an important public health issue; **preeclampsia is among the leading global causes of maternal and infant death**. The World Health Organization (WHO) estimates that preeclampsia occurs among 3.2% of live births globally resulting in approximately 4 million cases annually with 72,000 being fatal among infants (29). Preeclampsia has detrimental effects on the infant such as an increased risk of stillbirth, preterm birth, growth restriction (30), and cerebral palsy (31) due to the limited oxygen and nutrient transfer during fetal growth (30).

Preeclampsia can be considered as a 2-stage disease; the first stage is the unsuccessful remodeling of the spiral arteries in the uterus (32). This is proposed to result in reduced placental perfusion, the root cause of preeclampsia. During normal pregnancy trophoblast cells invade and remodel the spiral arteries of the placenta to make the artery walls thinner. With absent smooth muscle, the lumen is larger resulting in reduced resistance to blood flow to the placenta (33). The second stage, the maternal syndrome, is proposed to be a consequence of inflammation with endothelial dysfunction which results in circulatory disturbances in the various organ systems of the mother, including the renal, hepatic and central nervous systems (34). This second stage

includes an interaction with maternal constitutional factors such as genetics, obesity or diet that leads to the systemic pathophysiological changes including hypertension and proteinuria in the mother (32).

1.2.1.2 Epidemiology of Preeclampsia

Preeclampsia is a pregnancy specific disorder, which is diagnosed by new onset proteinuria and gestational hypertension (increased blood pressure) occurring after 20 weeks gestation. Preeclampsia is different from gestational hypertension which is recognized as blood pressure >140/90 mmHg for the first time after 20 weeks of gestation, without proteinuria in previously normotensive women (35). Offspring of preeclamptic women have a 5-fold increased risk of mortality compared to non-preeclamptic women (36). **Several studies suggest that there is at least a two-fold increased risk of fetal growth restriction (FGR) among infants of preeclamptic women compared to normotensive women.** (21, 23, 24). Reduced placental perfusion is a characteristic of both FGR and preeclampsia. However only about a third of infants from preeclamptic pregnancies are growth restricted (22). It is postulated that the metabolic changes of preeclampsia represent an appropriate response of the fetus to reduced placental perfusion(37). Metabolic changes are absent in FGR pregnancies without preeclampsia. Therefore it is theorized that FGR occurs in preeclamptic cases when the metabolic adjustments are inadequate to overcome profoundly reduced placental perfusion (33, 38).

1.2.1.3 Preterm Preeclampsia

Preterm preeclampsia is defined as preeclampsia that results in delivery prior to 37 weeks of pregnancy while term preeclampsia is preeclampsia with delivery at 37 weeks or beyond (39). Preterm preeclampsia is associated with a greater maternal and fetal morbidity (40), as well as

higher rates of recurrence of the disease and higher rates of small for gestational age infants compared to term preeclampsia (41). Furthermore, later life cardiovascular disease is more common in women with preterm than term preeclampsia (42). Our study will examine preterm and term status separately as these findings imply that it is important to stratify preeclampsia by preterm and term status.

1.2.1.4 Risk factors for Preeclampsia

Extensive research indicates that there are several important risk factors for preeclampsia. The following table summarizes data from multiple studies that identify the common risk factors for preeclampsia (Table 1). Preeclampsia in a previous pregnancy (OR range 5.0-21.5) and pre-existing hypertension (OR range 4.45-8.97), are among the strongest risk factors for preeclampsia. Obese and overweight women have a 2 to 5 fold increase in the risk of having a preeclamptic pregnancy. Obesity as a risk factor for preeclampsia is a major public health concern due to the global epidemic of obesity. The Centers for Disease Control (CDC) reports that over a third of adults in the United States (over 72 million) were obese in 2005-2006 and that 35.3 percent of women are obese (43). Older maternal age and pre-existing diabetes are moderately strong risk factors accounting for approximately two- to five-fold increase in the risk for preeclampsia. It is proposed that vascular endothelial damage is a natural process of aging and plays a role in increasing the risk of preeclampsia among women over 40 years of age (44). Nulliparity vs. multiparity triples the risk for preeclampsia. The increased risk of preeclampsia among first pregnancies (44-48) is consistent with the hypothesis which suggests that an immune maladaptation response towards invading cells of the fetus may influence preeclampsia(49). This

maladaptation is reduced by exposure to paternal antigen as occurs with the fetal maternal hemorrhage that normally accompanies delivery(50).

Maternal cigarette smoking during pregnancy is associated with adverse outcomes such as increased risk of spontaneous abortion, low birth weight, preterm birth, fetal growth restriction and perinatal death (51, 52). Paradoxically however, the risk of preeclampsia is lower among smokers compared to non-smokers (53-55).

The etiology of the Black-White racial disparities in adverse pregnancy outcomes is not fully understood (56). Black race is associated with an increased risk of preeclampsia (45) (57) as well as related severe complications (58). In addition, infants born to Black mothers are smaller and have higher rates of growth restriction as well as preterm delivery compared to infants born to White mothers (59-62). Adjustment for social and maternal risk factors does not fully explain the racial disparity in adverse pregnancy and birth outcomes (63, 64).

Table 1: Selected Studies of Common Risk factors of Preeclampsia and Fetal growth Restriction

Risk Factors	Preeclampsia	Comparison group	Reference
History of preeclampsia*	21.5 (9.8-47.2) [a]	Non-preeclamptic women	(46)
	11.2 (9.0-14.0)[a]	Women with no prior preeclampsia	(65)
	6.3 (4.4-9.2) [a]	Normotensive women	(44)
	5.0 (1.7-17.2) [a] (severe)	Women without severe preeclampsia	(47)
	7.2 (2.74-18.74) [a] (severe)	Normotensive women	(57)
Pre-existing hypertension	8.97 (8.06-9.99)[c]	Women without chronic hypertension	(66)
	4.4 (2.8-4.1)[b]	Women without chronic hypertension	(65)
	1.99 (1.78-2.22) [c]	Women without chronic hypertension	(67)
Pre-pregnancy BMI (kg/m^2)	7.6 (4.2-13.7)[c] (severe)	BMI=35kg/m^2 vs. BMI=20kg/m^2 White women	(68)
	5.3 (2.9-9.7)[c] (severe)	BMI=35kg/m^2 (Black) vs. BMI=20kg/m^2 (White)	(68)
	3.5 (1.68-7.46) [a]	BMI ≥32.3 kg/m^2 vs. <32.3 kg/m^2	(57)
	2.8 (1.4-5.7)[b]	BMI =35 kg/m^2 vs. 21 kg/m^2	(69)
	2.6 (2.3-2.9)[b]	BMI ≥30 kg/m^2 vs. <25 kg/m^2	(65)
	2.4 (1.8-3.1) [a]	BMI >24.2 kg/m^2 vs. 19.8-24.2 kg/m^2	(44)
Maternal Age ≥40 years	5.48 (1.62-18.5)[a]	Vs. women 20-30 years	(70)
	4.39 (2.05-9.37) [c]	Vs. women 20-30 years	(70)
	1.9 (1.2-2.9)[b]	Vs. women 20-29 years	(71)
	1.8 (1.3-2.6) [a]	Vs. women 20-29 years	(71)
Pre-existing Diabetes	5.58 (2.72-11.43) [b]	Type 1 vs. Non diabetic women (<35 years)	(72)
	2.77 (2.22-3.47) [c]	Non diabetic women	(66)
	2.1 (1.4-3.0)[b]	Non diabetic women	(65)
Nulliparity	3.9 (3.0-5.2)	nulliparous vs. multiparous	(45)
	3.6(2.6-5.0)	nulliparous vs. multiparous	(46)
	3.8 (1.7-8.3) (severe)	nulliparous vs. multiparous	(47)
	1.3 (1.2-1.5)	nulliparous vs. multiparous	(44)
Black Race	3.59 (1.54-8.38) [a]	Black/Hispanic vs. White women	(57)
	2.0 (1.2-3.4)[c]	Black vs. White women	(45)
Smoking in pregnancy	0.6 (0.50-0.60) (mild)[b]	Daily vs. nonsmokers at 1st antenatal visit	(54)
	0.5 (0.50-0.60) (severe)[b]	Daily vs. nonsmokers at 1st antenatal visit	(54)
	0.51 (0.44, 0.58) [c]	>9 cigarettes/day vs. no tobacco use	(73)
	0.84 (0.78, 0.91)[c]	Ever vs. never smoked during pregnancy	(74)

Adjusted relative risk or odds ratio (95% confidence interval) *History of preeclampsia in a prior pregnancy
[a] Multiparous women [b] Nulliparous women [c] Nulliparous and multiparous women

1.2.2 The Influence of Sex on disease

1.2.2.1 Epidemiological studies of the influence of Sex upon Disease

Emerging evidence suggests that the frequency, expression and outcome of illnesses in adults is different based on sex (12). For example, the risk of a heart attack is greater among men; however women have an increased risk of dying within a year of an attack (13). Males have poorer rates of survival from various cancers (14-16) as well as higher rates of Parkinson's disease (17, 18) compared to women. Several mechanisms, including sex steroids affect differential sex outcome. Sex steroids play a role in the expression and outcome of diseases such as heart disease. Both testosterone (75-79) and estrogen (78, 80, 81) promote vasodilation of blood vessels and influence vascular function. Low testosterone levels predict stroke (82), ischemic attack (82) and cardiovascular mortality (83, 84) in older men, while the large Framingham study reported that the rate of cardiovascular disease increases after menopause in women (85). This suggests that sex hormones may play a role in the pathogenesis of cardiovascular and other later life diseases. Based on this evidence, we posit that differential risk by sex for disease exists as early as in uterine development.

1.2.2.2 Fetal Sex and Pregnancy Outcome

The majority of the current literature suggests that males are at a disadvantage at birth (Table 2). As a result, male excess morbidity at birth is of concern in the global medical community (86)**.** A retrospective study of 75,725 births in a United Kingdom hospital found that spontaneous preterm birth is more common among women carrying male fetuses than when carrying female fetuses (9). A retrospective study in Dublin of 8,075 births among first pregnancies, reported that

women who experience spontaneous preterm labor as well as women who deliver at term are more likely to encounter complications requiring mechanical/instrument assistance if the infant is male (87). Other large studies show that complications during labor such as failure to progress (10, 88), true knots of the umbilical cord (88, 89), low Apgar scores (11, 88), fetal distress (11, 90, 91) as well as higher rates of perinatal mortality (11, 91) are more common among male than female infants. The literature review revealed one study which reported no difference by sex for adverse outcomes among FGR infants, however this study was underpowered to detect the odds ratios reported in the previously cited studies (92). Many studies on the male disadvantage fail to separate the morbidities based on physical/mechanical or a biological disadvantage. Male infants are on average larger and heavier than female infants. This explains complications in pregnancy related to longer periods of labor and instrument or operative delivery. However the larger size of male infants does not explain the greater incidence of preterm birth, fetal distress or death. This suggests that other factors related to the sex of the infant may explain this sexual dimorphism.

1.2.2.3 Potential Biological Mechanisms

Little is known about the biological mechanisms responsible for the differential adverse outcomes in pregnancies by sex. However, a few mechanisms including gene and steroid pathways have been proposed. *Gene pathways:* Male infants have a XY chromosome while females have a XX chromosome. Among females, one X is inactivated during embryogenesis. The chorionic villi of the placenta have the ability to reactivate the inactive X-chromosome (93). The reactivated X-chromosome is theorized to play a role in increasing the survival of female fetuses in compromised uterine environments through conservative reduction in placenta growth

that is insufficient to cause fetal growth restriction (94). Gene expression of immune receptors in the placenta has been found to vary by fetal sex. This is believed to play a role in the differential response to inflammation or infection as well as growth and development by fetal sex (95). *Steroid pathway*: Fetal glucocorticoids play a role in fetal organ development (96). Exogenous glucocorticoids administered to women at risk for preterm delivery reduce the risk of neonatal respiratory distress and accelerate tissue maturation (96). However, high levels of glucocorticoids are harmful to the fetus. Placental 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) metabolizes glucocorticoids. Stark and colleagues performed a study on women at risk for preterm delivery who were treated with the glucocorticoid betamethasone. He found a sex specific autoregulation of 11β-HSD2 among infants born within 72 hours of treatment with betamethasone. He hypothesized that the placental adjustment (suppression) of the 11β-HSD2 response among female infants decreased the metabolism of betamethasone. This allowed for the greater prophylactic effect of betamethasone among female infants. The adjustment of the response to glucocorticoids in female and the lack of adjustment in male infants may explain the greater morbidity and mortality observed among male infants (97).

1.2.2.4 Differential Outcome by Fetal Sex in Response to in utero Insults.

There is evidence that in response to in utero insults, male vs. female infants are at a greater disadvantage in pregnancy outcome. Bracero performed a study of 107 pregnant diabetic women found that male infants had higher morbidity than female infants at birth. Males had more hypoglycemia ($p=0.01$) and longer stays in intensive care ($p=0.01$) than female infants (98). Despite the fact that the Bracero study adjusted for maternal race, it failed to report data on the race of the mothers. Maternal race is known to influence fetal outcome. Another recent study of

323 diabetic women reported that male offspring of diabetic women were 3.5 times more likely to have a congenital malformation than female infants (OR 3.5 (95% CI 1.3–10.0); P = 0.02) (99). In a Swedish population study, 4749 women were diagnosed with chronic hypertension. These women had a 3 fold increase in intrauterine death (OR 3.07 (95% CI 2.12-4.46) compared to normotensive women, if the offspring was male. Female infants had no increased risk of intrauterine death (OR 0.98 (95% CI 0.51–1.89) (100).

Murphy found that among pregnant asthmatic women who did not use inhaled steroid for treatment of asthma, the female fetuses showed greater reductions in birthweight (p=0.02), while the growth of the males were unaffected (p=0.19) (101). However, among pregnant asthmatic women with severe exacerbations that required hospitalization, male infants had greater reductions in birth weight ($p<0.05$)(102). This is consistent with the theories proposed by Clifton(94) and Eriksson(103) that when faced with maternal insults, males maximize continued fetal growth while females reduce growth to increase chances of survival. However when faced with continued or increased in utero insult, males are at greater risk for adverse outcome because they have exhausted their placental reserves (94, 103). The Zetterstrom, Evers and Murphy studies were limited by little ethnic diversity in the study populations. Our study will analyze if the influence of fetal growth is modified by infant sex and race in a large Black and White population.

Preeclampsia is associated with an intrauterine enviroment that increases the risk of growth restriction and stillbirth (36). A case control study of premature deliveries at <32 weeks, at a university hospital reported that male fetuses were more likely to be associated with placental lesions that had chronic inflammation. This inflammation is indicative of a maternal immune response against the invading interstitial trophoblast (104). Therefore we propose that

the uterine insult in preeclamptic pregnancies may subject the already disadvantaged male offspring to a greater risk of growth restriction compared to female offspring.

1.2.2.5 Differential Outcome by Fetal Sex in Preeclampsia.

There is also evidence suggesting that there are differences in the outcomes of preeclamptic pregnancies depending on offspring sex. In contrast to the general population (105-107) where male fetuses are more common in preterm deliveries than at term, at least two studies indicate that male offspring were less common among preterm preeclamptic pregnancies, than among term preeclamptic pregnancies (106, 108).

Two small studies by Stark and colleagues found vasodilation and blood flow differences in preeclamptic women and their term offspring compared to normotensive women and their term offspring. The first study reports that the response to corticotrophin releasing hormone (CRH) was different in preeclamptic women with male but not female fetuses: the preeclamptic women bearing male offspring had reduced vasodilation in their peripheral microvascular and skin tissues while there was no difference among preeclamptic women bearing female offspring (109). In the second equally small study of 38 offspring of normotensive and 33 offspring of preeclamptic women, blood flow in the peripheral microvascular and skin tissues was different in male and female infants of preeclamptic mothers after birth (110). In both studies some of the preeclamptic women received anti-hypertensive medication which may have influenced microvascular function. However, the equal numbers of male and female infants exposed to the medication reduced the bias introduced by the medication in interpreting the influence of fetal sex on microvascular function. Because the systemic vasculature of both the fetus and mother is

influenced by fetal sex, we also suspect that fetal sex may influence the placental vasculature, shape of the placenta, and fetal grow in the setting of preeclampsia.

There is evidence to suggest that fetal sex affects maternal blood pressure, and hemoglobin concentration**.** Early gestational blood pressure was significantly higher in a large study of women who had gestational hypertension bearing male offspring than women with female offspring. Women with gestational hypertension also had lower third trimester hemoglobin values and less frequent proteinuria if their offspring was male. Surprisingly, however, Naeye reported that there were fewer syncytial knots (placental result of low uteroplacental blood flow) among the placenta of male fetuses (111). The Naeye study defined preeclampsia as 2 or more diastolic blood pressures that exceeded 90mmHg. This definition is more consistent with the definition of gestational hypertension than preeclampsia in our study and was re-defined for the purposes of this literature review as gestational hypertension. Increased blood pressure and gestational hypertension are signs of preeclampsia. These combined with low hemoglobin levels suggest that male sex may influence preeclampsia.

Differential fetal sex outcome is not a well-studied area. To our knowledge, there are no previous studies that have investigated differential growth by fetal sex in preeclampsia. Our study is specifically designed to expand this area.

Table 2: Summary Table of Selected Studies showing that Women with Male Fetuses are at Greater Risk for Perinatal Complications.

Study population	Males vs. Females		Reference
423,033 singleton pregnancies in the Netherlands	**Increased risk of:** Fetal Distress during labor Apgar score at 5 min ≤3 Perinatal death	 1.48 (1.44-1.51) 1.27 (1.20-1.34) 1.27 (1.20-1.34)	(11)
75,725 deliveries in UK teaching hospital over 11 years	**Increased risk of:** Preterm delivery Spontaneous preterm delivery	 1.13 (1.06-1.20) 1.30(1.19-1.42)	(9)
1,158,276 infants born in Sweden 1990-2001	**Maternal Risk of:** Preeclampsia> 28 wks GA	 1.02 (1.00-1.04)	(108)
55,891 male and 53,104 female neonates in Negev Israel 1988-1999	**Increased risk of:** Vacuum delivery Apgar at 1 min <7 Birthweight>4kg Failure to progress to *2nd stage	 1.5 (1.4-1.6) 1.3 (1.3-1.4) 2.0 (1.8-2.1) 1.4 (1.3-1.5)	(88)
4 datasets and 20 populations	**Increased risk of Preterm birth <37 weeks:** EUROPOP SSD Registry FNPS	 1.14 (1.06-1.22) 1.14 (1.03-1.27) 1.13 (1.01-1.27)	(107)
866,188 women with singleton pregnancies from the Swedish Medical Birth Registry 1992–2004.	**Males of chronic hypertensive mothers vs. normotensive had increased risk of:** Intrauterine death Neonatal death	 3.07 (2.12-4.46) 2.99 (1.84-4.85)	(100)
Adjusted relative risks or odds ratio (95% CI) reflect males vs. females *2nd Stage= dilation of cervix to delivery of infant.			

1.2.3 Fetal Growth

1.2.3.1 Normal Fetal Growth

Fetal birthweight is a key predictor of perinatal outcome (112). The fetus achieves only 10% of its final birth weight in the first 20 weeks of gestation. During the first half of pregnancy fetal development is directed at organogenesis rather than growth. Around 28 weeks of gestation

exponential fetal growth begins and is accompanied by fetal weight gain of approximately 200g/week(113).

1.2.3.2 Determinants of Fetal Growth

Several modifiable and non-modifiable risk factors that influence fetal growth are presented in the table below (Table 3). These illustrate that the study of preeclampsia is complex based on the fact that several risk factors common to preeclampsia and fetal growth restriction behave differently in each setting. Similar to risk factors for preeclampsia (Table 1), older maternal age and black race are associated with an increased risk of fetal growth restriction (Table 3). Biologic determinants such as race reflect the genetic environment of the fetus. Infants born to Black mothers are smaller and have higher rates of growth restriction as well as preterm delivery compared to infants born to White mothers (59-62).

However, there are other risk factors that show an influence in the opposite direction for preeclampsia compared to growth restriction. Maternal smoking during pregnancy is a modifiable risk factor that increases the risk of fetal growth restriction two to threefold compare to non-smokers. Paradoxically however, the risk of preeclampsia is lower among smokers compared to non-smokers. Pre-pregnancy BMI and pregnancy weight gain are important modifiable risk factors that impact fetal growth. As maternal BMI increases the risk of fetal growth restriction decreases and birth weight increases. Underweight mothers and normal-weight mothers are more likely to give birth to growth restricted infants, while obese mothers are likely to give birth to large for gestational age infants. However, obesity is associated with an increased risk of preeclampsia.

Table 3: Selected Studies of Risk Factors for Fetal Growth Restriction and Preeclampsia

Determinants of Growth	Growth Restriction	Comparison group	Reference	Excess Growth (LGA)	Comparison group	Reference	Risk for Preeclampsia	Comparison group	Reference
Same Direction									
Maternal Age	2.09 (NR) 1.3 (1.0-1.6) 1.13(1.06-1.20)	>40 vs.20-29 years >35 vs. 20-34 years >35 vs. 21-25 years	(114) (59) (115)				5.48 (1.62-18.5) 4.39 (2.05-9.37) 1.9 (1.2-2.9) 1.8 (1.3-2.6)	≥40 vs.20-30 years [b] ≥40 vs.20-30 years [b] ≥40 vs.20-29 years[a]	(70) (70) (71) (71)
Black Race	2.6 (1.8-3.7) 1.4 (1.1-1.7)	White Non-Hispanic White	(116) (59)	2.8 (2.2-3.8) risk of LGA among preterm infants	Non-Hispanic White	(59)	2.22 (1.37-3.62) [s] 2.0 (1.2-3.4)	Black/Hispanic vs. White[c] Black vs. White [c]	(57) (45)
Opposite Direction									
Maternal Smoking	3.31 (3.19-3.44) 2.89 (2.00-4.20) 2.87 (2.24-3.68) 2.6 (2.2-3.2) 2.28(2.14, 2.43)	Smoke in pregnancy vs. no Each 25 cig/day increase Smoker: yes/no 10+/day vs nonsmoker Smoker at 1st antenatal visit vs. nonsmoker	(115) (52) (117) (59) (51)				0.73 (0.71-0.75) 0.5 (0.50-0.60)	Smoker vs. nonsmoker Smoker vs. nonsmoker (at registration)	(118) (54)
Gestational weight gain							1.54 (1.46-1.63)	<41lbs vs. ≥41 lbs	(66)
Maternal Pre-pregnancy Body Mass Index (BMI)	1.69 (NR)[c]	<18.3 vs 18.3-28.8kg/m^2 [c]	(114)	1.66(1.20-2.30) 1.04(1.01-1.08)	≥25 vs. <25kg/m^2 >30 vs. 18.5-25 kg/m^2	(119) (120)	7.6 (4.2-13.7)[c s] 5.3 (2.9-9.7)[c s] 3.5 (1.68-7.46) [b] 2.8 (1.4-5.7)[a] 2.6 (2.3-2.9)[a] 2.4 (1.8-3.1) [b]	35 vs. 20kg/m^2 (White) 35(Black) vs.20kg/m^2 (White) ≥32.3 vs. <32.3 kg/m^2 35 vs. 21 kg/m^2 ≥30 vs. <25 kg/m^2 >24.2 vs. 19.8-24.2 kg/m^2	(68) (68) (57) (69) (65) (44)

NR= Not Reported,
[a] primiparous women
[b] multiparous women
c primiparous and multiparous women
[s] severe preeclampsia

1.2.3.3 Abnormal Fetal Growth

Normal fetal growth is essential for appropriate fetal outcome following pregnancy. It is well recognized that abnormal growth at both extremes of the fetal weight distribution is associated with an increased risk for perinatal morbidity and mortality. As a result, abnormal growth is a major public health issue. These adverse events include necrotizing enterocolitis, and respiratory distress syndrome in reduced growth cases (121), while trauma, fractures and facial paralysis can occur in cases with large for gestational age infants (122). Fetal growth restriction (FGR) is defined as the failure of fetus to grow to his/her genetic growth potential (1). A recent study that compared 3 definitions of FGR revealed that FGR infants have a 2.5-3.5 fold increased likelihood of fetal death, and a 1.5-2.0 fold increase in the likelihood of requiring assisted ventilation 28 days after birth, regardless of the definition (2). Fetal growth restriction is also associated with a two fold increase in the likelihood of an infant with cerebral palsy (OR 2.3 (95% CI 1.8–3.0) (123). Additionally, fetal growth restriction is associated with consequences for the fetus later in life (3). These include an increased risk of hypertension, cardiovascular disease and mortality (124), and type 2 diabetes mellitus (125). Growth restriction early in pregnancy results in a symmetric reduction in length, weight and head size. This is consistent with the genesis of growth restriction at this time, which is related to viral infection and congenital abnormalities resulting in a net decrease in cell number. Restriction later in pregnancy is associated with reduced nutrient availability for the fetus and results in reduction in weight, length, fat mass and is often accompanied by "brain sparing" growth (normal head circumference) (126) except in the most severe cases in which compensatory mechanisms are overcome and all organ growth is reduced (127-129). SGA is a reasonably effective indicator of

fetuses that are at risk for perinatal morbidity and mortality. However studies of growth have been limited by the analysis of only SGA as the sole marker of fetal growth restriction, and it is possible that the impact of preeclampsia on fetal growth has been underestimated. It is essential to therefore investigate additional indicators of fetal growth to determine the combined effect of sex and preeclampsia on growth.

1.2.3.4 Fetal Growth and Adult Disease

The Fetal Origins of Adult disease hypothesis suggests that reduced fetal growth is associated with offspring risk for cardiovascular and other chronic diseases later in life (130). Barker proposed that in response to a reduced nutrient supply the fetal system alter metabolic processes and organs to adapt (fetal programming). He theorizes that this fetal programming is permanent and predisposes the infant to heart disease and diabetes in adulthood (130). Barker also posits that over-nutrition of these infants post-partum may also further contribute to later adult disease based on the increased demand on the reduced functionality of organs such as the kidney and heart (131). Ensuing follow-up studies by Barker and other researchers have provided evidence to support the hypothesis: Low birth weight and reduced fetal growth is associated with an increased risk of cardiovascular disease (5), hypertension (6) and Type 2 diabetes (7, 8) in later life. Based on the Developmental Origins of Adult Disease hypothesis, a compromised uterine environment in the presence of preeclampsia may be a mechanism that predisposes the infant to disease later in life. Observational human studies support this theory: Kajantie analysed risk for hypertension and cardiovascular disease among adult offspring of 284 preeclamptic women who were enrolled in the Helsinki Cohort Study, and found that the risk of hypertension was increased by 50% (RR 1.5; 95% CI 1.1-2.3), while the risk of stroke increased 2-fold (RR 2.2;

95% CI 1.2-4.1) among offspring of women with severe preeclampsia compared to offspring of normotensive women (132). A second study investigated the day and night ambulatory blood pressure (ABP) among 57 age and sex matched pairs of 12 year old offspring of preeclamptic and normotensive pregnancies. This study found that for both night and day time readings, the offspring of preeclamptic women had significantly higher mean 24-hour systolic and diastolic ABPs compared to the offspring of normotensive women. (133).

A few researchers have challenged the fetal origins of adult disease hypothesis. They cite statistical errors or inappropriate adjustment for current weight and confounders as flaws in the interpretation in studies of the relationship between birthweight and later life disease (134-136). However, evidence from animal studies of fetal programming provides biological support for the fetal origins of adult disease hypothesis. These animal studies also show sex differences in response to fetal programming. Alexander investigated if FGR rats from induced placental insufficient pregnancies showed elevated mean arterial pressure. She found that induced placental insufficiency in late pregnancy resulted in elevated mean arterial pressure in male and female growth restricted rats (137). However at 12 weeks the blood pressure of the female offspring became normal (i.e. blood pressure was not significantly different from control female rats) while the male offspring remained hypertensive (137). A study by Woods et.al investigated the effect of moderate protein diet restriction during pregnancy of rats on mean arterial pressure of adult offspring. The study found a greater increase in blood pressure and vascular abnormalities in peripheral arteries of male but not female offspring (138). In addition to this finding, severe protein restriction results in hypertension and changes to the structure of the kidney in both male and female adult offspring (139, 140). These animal studies suggest that

male infants may be more susceptible to in utero insults than female infants to the maternal environment during pregnancy and that the results of fetal programming differ by infant sex.

Based on evidence from these observational and animal studies, it is important to understand the mechanisms by which fetal growth restriction and preeclampsia are associated with long term offspring risk for cardiovascular morbidity and mortality. Human observational studies have not commonly evaluated the effect of infant sex on their findings. Studying the influence of sex on the health of offspring of preeclamptic women may provide early markers of individuals who should be targeted for interventions before clinical manifestation of later life disease.

1.2.4 Additional Indicators of Fetal Growth

Small for gestational age (SGA) is defined as birthweight below the 10th percentile for babies of the same gestational age from the internal or an external referent population and is often used to measure FGR. SGA can be adjusted for factors such as race, infant sex and parity(141). Kramer studied 11.5 million Black and White singleton live births greater than 22 completed weeks of gestation and birthweight greater than 500 g, in the United States from 1998 to 2000(142). He found that the risk of SGA among Blacks vs. White infants was 2-fold when he used a non-race specific standard (HR 2.05; 95% CI 2.04-2.06) and that the greater risk of SGA among Black infants was lost when he used a race-specific standard (HR 1.05; 95% CI, 1.04-1.06)(142). Given the well-established lower mean birthweight and higher mortality rate among Black vs. White infants, it is reasonable to expect that growth restriction and not physiology contributes to these higher rates in Blacks. Therefore in our study we will not adjust the SGA charts for race.

Because there are limitations of SGA to determine impaired fetal growth it is helpful to utilize additional growth indicators such as ponderal index (PI), head to chest circumference (HCC) ratio and the fetal placental ratio (FPR) to further characterize fetal growth. We will utilize these markers along with SGA to characterize growth in normal pregnancy as well as pregnancies complicated by preeclampsia to assess if growth is modified by fetal sex.

1.2.4.1 Ponderal index

Ponderal Index provides an estimate of relative thinness of the fetus. It is defined as Weight (gm)/Length $(cm)^3$ x 100. A low ponderal index is an indicator of asymmetric fetal growth. Ponderal index increases with gestational age. Ponderal index values of < 2.0 between 29 and 37 weeks and <2.25 beyond 37 weeks are indicative of fetal growth restriction (143) (Table 4). Offspring of early onset preeclamptic women have lower birthweight and lower ponderal indices compared to offspring of normotensive women. However in late onset preeclampsia there was no significant difference between the preeclamptic and normotensive offspring (144) There is evidence to suggest that the growth of the chorionic plate area affects fetal body proportion: ponderal index appears to be directly proportional to chorionic plate area, however disc thickness did not appear to have an effect on ponderal index (27). Our study will initially characterize ponderal index by race and sex and gestational age at birth in uncomplicated pregnancies. We will then be able to compare normal fetal growth to fetal growth in pregnancies complicated by preeclampsia and determine if these associations are modified by offspring sex.

Contribution to understanding fetal growth: Ponderal index will be able to identify cases of growth restriction than may have been missed using birth weight for gestational age alone(145). A study which compared the traditional definition of FGR (birthweights $<10^{th}$

percentile) to low ponderal index (defined as PI below the 10th percentile after 36 weeks of completed pregnancy) found that the rates of fetal distress and caesarean deliveries were significantly higher in the low PI group compared to the SGA group (p=0.02 and p=0.0 respectively). This study confirmed the finding of 3 previous reports which suggest that PI may provide additional information in identifying cases of FGR than birth-weight percentiles alone (145-147). Ponderal index measures proportionality of skeletal to soft tissue growth. It indicates those infants who attained insufficient or excess soft tissue growth relative to the skeletal growth. Used in conjunction with SGA, PI will be a valuable contribution to understanding fetal growth. In SGA cases, the ponderal index is proposed to distinguish between inappropriate and appropriate soft tissue to skeletal growth. In cases where the weight is appropriate for gestational age, but the infant is disproportionate in length for given weight and age, the ponderal index would be able to detect that the infant has inappropriate soft tissue to skeletal growth which is an indicator of abnormal growth.

1.2.4.2 Head circumference to chest circumference (HCC) ratio.

HCC is defined as the head circumference in centimeters divided by the chest circumference in centimeters. HCC is an indicator of growth restriction (Table 4). **The underlying theory is that in the presence of growth restriction, visceral organ growth is compromised earlier and brain growth is compromised later. As a result HCC is indicative of symmetric or asymmetric growth** (148)**.** In normal growth HCC is inversely proportional to gestational age. Ounsted et.al. found that among SGA infants, males had higher ratios than females, while first born infants and offspring of preeclamptic women had significantly higher ratios compared to

SGA infants of normotensive mothers (149). Women with severe preeclampsia are at greater risk of delivering an asymmetric fetuses compared to a symmetric fetus (150).

The previously mentioned Stark paper which analyzed peripheral microvascular blood flow in offspring of preeclamptic women also analyzed infant symmetry. They reported that female fetuses exhibited asymmetric growth accompanied by reduced birth weight, while the male fetuses maintained symmetric growth in the presence of preeclampsia (110). However the study failed to report how symmetry was assessed. Our study will characterize HCC ratio and ponderal index by race and sex and SGA in 9,551 uncomplicated pregnancies and thus be able to compare growth in normal versus preeclamptic pregnancies.

Contribution to understanding fetal growth: HCC can be used to categorize infants as symmetric or asymmetric (disproportionately lagging in abdominal growth) in order to categorize the type of reduced growth (151). Small fetuses that are symmetrical may be small because they are normally grown (constitutionally small) or due to severe growth restriction in which the growth of the head has slowed to match the abdominal circumference. Symmetrically small infants are regarded as less common (150) and are considered to have worse perinatal outcomes compared to asymmetric growth restricted infants (150, 152). However two studies suggest that asymmetric growth restriction is associated with an increased risk of neonatal morbidity compared to symmetric SGA infants if congenital anomalies are removed (151, 153). The lower rates of neonatal morbidity among symmetric SGA infants without anomalies, suggests that this group also includes constitutionally small infants. Similar to PI, HCC will also be better able to identify growth restriction than using birth weight for gestational age alone. Used in conjunction with SGA, HCC will not only be able to confirm SGA, but will also determine if fetal growth was symmetric or asymmetric. In cases where the weight is appropriate

for gestational age, but the infant is disproportionate in the circumference of the chest relative to that of the head for the given gestational age, the HCC would be able to detect that the infant has compromised organ growth relative to brain growth which is an indicator of abnormal growth.

Table 4: Summary of Anthropometric Markers of Growth.

Fetal Marker	What it indicates	What indicates Growth Restriction
Small For Gestational Age (SGA)	FGR	<10th percentile for gestational age
Ponderal Index (PI)	Symmetric/Asymmetric Growth	< 2.0 between 29 & 37 weeks of gestation <2.25 beyond 37 weeks of gestation
Head to chest circumference (HCC)	Symmetric/Asymmetric Growth	Elevated (Normal is approx 1.0)

1.2.5 Placental Growth

1.2.5.1 Placental Function and Growth.

The placenta is the main source of nutrient supply to the fetus. It acts as a nutrient and waste exchange system. The placenta supplies the fetus with nutrients from the blood of the mother and transports waste products to the blood of the mother (154). Normal placental growth and function is essential for growth of the fetus. Studies show that abnormal placental growth is associated with increased risk of morbidity and mortality and adverse lifelong consequences such as mental, visual and hearing impairment, autism and cerebral palsy (155, 156).

The growth of the placenta is an important factor in the growth of the fetus. Fetal growth is controlled by the nutrient supply of the mother and the placenta. In pregnancy, maternal blood is delivered to the placenta via the spiral arteries, where it bathes the chorionic villi in the intervillous space. The fetus sends blood to the placenta via two umbilical arteries. The fetal arteries branch within the chorionic villi providing a large surface area for nutrient and waste exchange with the maternal blood (157). Placental growth follows an S-curve (158). The rate of growth of the placenta is greater than fetal growth in the first 16 weeks of pregnancy resulting in the weight of the placenta being greater than the fetal weight during this period. However the placental and fetal weights are similar between 17 and 28 weeks. At approximately 27 weeks of pregnancy the placenta weighs approximately 50% of its term weight while the fetus has only achieved 30% of its term weight. During maximum fetal growth (28-38 weeks) the rate of growth of the placenta is lower than the fetal growth and is observed to decelerate after 38 weeks (113). The placenta plays a crucial role in supplying the fetus with nutrients as well as producing growth hormones. These hormones for e.g. insulin-like growth factors (IGF-1 and IGF-2) which plays a major role in controlling growth of fetus and placenta and vascular endothelial growth factor (VEGF) which is responsible for proliferation of trophoblasts and vasculogenesis are important for fetal and placental growth(113).

1.2.5.2 Compensatory Mechanism of the Placenta

Understanding the growth of the placenta is essential in understanding the growth of the fetus. Studies indicate that term offspring of preeclamptic women have had fetal growth similar to offspring of normotensive women (110, 159, 160). Molteni suggests that diseases such as preeclampsia can inhibit proper placental growth by limiting placental blood flow, and this may cause the placenta to slow its growth progression. He suggests however that fetal weight may be

maintained provided that there is sufficient placental reserve to sustain fetal growth (161). The placenta can survive the functional loss of 30-40% of the placental villi without compromising fetal growth and also has the capacity for compensatory growth (4). As a result it is imperative to investigate the growth of the placenta as fetal weight may be sustained while the placenta is compromised. Even with apparent normal fetal growth, the compromised placental growth and its adaption to the restrictive environment may result in fetal programming which places the infant at risk for disease later in life. Compromised placental growth is indicative of underlying risk factors for disease later in life such as hypertension (6, 162), (as discussed in previously in the fetal growth section). Eriksson and colleagues reported that hypertension in men was associated with a shortened smaller diameter of the placenta. However, hypertension in women was associated with a small placental area (103). In addition, they also found that at birth, males had larger placentas compared to females. However, the male placentas were smaller in comparison when compared to birthweight and may indicate lower reserve capacity (103). The study cohort was born during the Second World War during a time of severe food shortages(163). This suggests that growth of the placenta in response to in utero insult or reduced nutrient supply is different based on infant sex. Our study will investigate the morphometric features of the placenta in order to better understand placental growth in offspring of preeclamptic women, and determine if this growth is modified by offspring sex. This can provide an early marker of persons who should be targeted for interventions before clinical manifestation of disease.

1.2.5.3 Determinants of placental growth

There are several risk factors associated with abnormal placental growth. Similarly to FGR and preeclampsia, Baptiste-Roberts reported that Black race is associated with an increased risk of placental growth restriction (growth that is $<10^{th}$ percentile of the respective placental growth measure). In the CPP, Black compared to White race was associated with a 66% increase in the likelihood of low placental weight (OR 1.66; 95 % CI 1.51-1.82), a 3-fold increase in the likelihood of small placental disk thickness (OR 3.22; 2.90-3.58) and a 36% increase in the likelihood of restricted growth of the chorionic plate area (OR 1.36; 95% CI 1.24-1.49). The study also found that hypertensive disease beyond 24 weeks of pregnancy increased the likelihood of growth restriction for the placental weight and chorionic plate area approximately 2-fold (OR 1.98; 95% CI 1.54-2.55, and OR 1.86 95% CI 1.46-2.37 respectively). Similar to FGR, Baptiste-Roberts found that each $1kg/m^2$ increase in pre-pregnancy BMI was associated with a reduced likelihood of low placental growth (164).

1.2.6 Markers of placental growth

Studies of placental growth are limited by their analysis of placental weight as the sole marker of placental growth (25, 162). The ratio of fetal weight to placental weight (FPR) is another marker that is used as a measure of the efficiency of placental growth relative to fetal growth (27, 112). The fetus and the placenta share the same genetic material (165) thus fetal weight relative to placental weight may be a better predictor of fetal and placental growth because it avoids the influence genetic factors. There are, however, limitations to this approach. Placentas that are large and thin as well as placentas that are small and thick can produce FPRs that are consistent

with normal proportioned placentas (27). These different placental proportions may result in functionally different placentas (28). Based on these limitations, it is advantageous to employ alternate placental measures. The larger and smaller placental diameters, ratio of the diameters, thickness and FPR, will be better able to ascertain placental growth beyond placental weight.

1.2.6.1 Fetal Placental Ratio

FPR increases with fetal growth and gestational age. Appropriate for gestational age (AGA, birthweight between 10^{th} and 90^{th} centiles) and large for gestational age (LGA, birthweight $>90^{th}$ centile), infants have consistent placental growth up to 42 weeks of gestation, while among small for gestational age infants (SGA) (birthweight $<10^{th}$ centile), placental growth tends to stop at around 34 weeks, but fetal growth may continue until term (161).

SGA infants tend to have lower fetal placental ratios (112, 161) (Table 5). When comparing FPR in uncomplicated pregnancies to pregnancies complicated by gestational hypertension or fetal growth restriction using LGA, AGA, and SGA categories, as expected, it was found that the FPR was smallest among the SGA group and largest among the LGA group (166). FPR in SGA pregnancies is lower and FPR in LGA pregnancies are higher than FPR in AGA pregnancies throughout gestation.

A low FPR (low fetal weight relative to placental weight) is suggestive of a less efficient placenta and occurs in impaired environments such as maternal anemia (167), maternal smoking, low socioeconomic status (168) and obesity (169). The work of Lao and Wong suggest that the reduced FPR among SGA infants is due to the combined result of continued placental growth (increase in placental size) and deceleration of fetal growth (170).

It is interesting to note that **high FPRs also are associated with growth restriction:** High FPRs are indicative of a small placenta relative to the fetal weight. Molteni points out that placental growth may stop at 34 weeks, however if there is sufficient placental reserve, then fetal growth may continue (161), and the FPR will be high. High FPRs suggests that the placental reserve has been exceeded and is unable to sustain optimal fetal growth (171) and place infants at hypoxic risk during delivery (161). FPRs greater than 10.35 are associated with the risk of fetal distress at birth or stillbirth (161, 172) (Table 5). **This indicates that it is important to analyze both extremes of the FPR distribution to ascertain growth.**

Table 5: Summary of reported mean fetal placental ratio (FPR) from selected Studies.

Study population	Mean FPR (SD)	Reference
11,141 uncomplicated single term pregnancies 37-42 weeks of gestation	**37 weeks** 5th percentile=4.27 50th percentile= 5.68 95th percentile=7.58 **40 weeks** 5th percentile= 5.03 50th percentile= 6.41 95th percentile=8.20	**(173)**
16,616 uncomplicated singleton pregnancies with 1569 small for gestational age (SGA) and 15,047 appropriate for gestational age (AGA) infants between 32 and 42 weeks	**SGA** 32 wks=4.68 36 wks= 5.71 40 wks= 6.93 **AGA** 32 wks=4.78 36 wks= 6.30 40 wks= 7.05	**(112)**
29,710 full term, 2675 SGA and 27,035 AGA infants from the collaborative perinatal project (CPP).	SGA =6.94 AGA=7.35	**(174)**
238 normal pregnancies, 36th-40th gestational weeks.	**Median** 36 weeks = 5.16 37 weeks=5.99 38 weeks=6.01 39 weeks=6.07 40 weeks= 6.20	**(175)**
High Fetal Placental Ratios		
417 low risk infants 38-42 weeks of gestation	FPR ≥11 = increased risk of fetal distress =increased risk of Agar score ≤6	**(172)**
Not reported.	FPR≥10.35 =increased risk of fetal distress =increased risk of stillbirth	**(176) as cited in (161)**
*GA= gestational age, SGA= small for gestational age, AGA = appropriate for gestational age, LGA=Large for gestational age		

1.2.6.2 Larger and Smaller Placental diameters.

Placental growth is expected to be centripetal around the umbilical cord (171, 177). The largest and smallest placental disk diameters determine the surface area available for spiral arteries from the uterine wall to perfuse the placenta (Table 6). Placentas that are wide in both diameters are in contact with more spiral arteries than a smaller disk (27). The extent of the lateral growth of the placenta on the uterine wall determines the number of spiral arteries that are available for conversion by the placenta (171).

Salafia suggests that a chorionic plate that is wide in both diameters (with a large vascular tree) that is thin (with fewer villus branching) may present a very different fetal cardiovascular burden than a chorionic disc that is small in surface area but very thick (28). A large retrospective study by Kajantie on subjects born in the Helsinki Birth Cohort (1934-1944) found that smaller placental surface area was associated with preeclampsia. When the larger and smaller diameters of the placenta were analyzed together, preeclampsia was strongly associated with the length of the smaller diameter, but not the larger diameter ($p<0.0001$). He also found a dose-response effect- the shorter the smaller diameter, the greater the risk and severity of preeclampsia (178).

A large retrospective study was performed by Misra using offspring from the Collaborative Perinatal Project (1959-1965). Misra found that the birthweight and fetal placental ratio of females compared to males was more sensitive to changes in the placental area for female vs. male infants (179). We posit that fetal programming of the placenta in the presence of preeclampsia will be different by infant sex. We propose that the size of the placenta reflected by the larger and smaller diameters (surface area) is an indicator of the nutrient supply to the fetus

and as a result can be used as a proxy to compare the available vasculature system that facilitates delivery of nutrients for growth between offspring of preeclamptic and normotensive women. The diameters should provide additional information on the vasculature in the cases where the placentas may be proportionate, large and thin, or small and thick. We propose and will test therefore that the diameters of the placenta will be related to preeclampsia and birth weight in a manner not solely explained by placental weight. We theorize that the relationship of birth weight to growth of the placental diameters will be different among offspring of preeclamptic women compared to offspring of normotensive women by infant sex. Understanding the influence of fetal sex on the relationship between preeclampsia and fetal growth may help to explain why morbidity and mortality rates differ by sex in pregnancy complications.

1.2.6.3 Ratio of the diameters

The ratio of the diameters indicates the asymmetry of placental growth (171), which is expected to be centripetal around the umbilical cord (171, 177) (Table 6). Placental underperfusion may cause early onset or excess blood flow to the placenta resulting in excessive oxidative stress, that leads to asymmetric placental shape(180). Asymmetric shapes may also be caused by reduced growth of the placental tissue during the second and third trimesters due to chronic oxidative and other stresses that may have been induced through underperfusion of the placenta(180).

In a previously mentioned study, Kajantie found a dose-response relationship between placental asymmetry and preeclampsia. As the placental became less symmetric (more oval-shaped) the association with preeclampsia increased (178). We theorize that the relationship of birth weight to growth of the ratio of the placental diameters will be different among offspring of preeclamptic women compared to offspring of normotensive women by infant sex.

Understanding the relationship between birth weight and asymmetry of the placenta in the setting of preeclampsia may bring us closer to understanding the pathophysiology of preeclampsia as well as mechanisms for sex differences in pregnancy outcome.

1.2.6.4 Placental disk thickness

The thickness of the placenta is an indicator of the villus volume available for nutrient transfer to the fetus (Table 6). The branching of the placental villi increases its thickness (27). Kajantie found that the mean thickness of placentas from women diagnosed with preeclampsia was greater than in normotensive pregnancies (178). This may indicate compensation for the smaller mean placental area found among the preeclamptic vs. normotensive women in his study (178). He also found that the relationship between disk thickness and birth weight was different in male vs. female infants. We propose that similarly to the smaller and larger diameters, the disk thickness will provide additional information about the vasculature in the cases where the placentas may be proportionate, large and thin or small and thick. We theorize that the relationship of birth weight to growth of the placental diameters will be different by sex among offspring of preeclamptic women vs. normotensive women. Based on the Barker hypothesis we theorize that these compromised cases may indicate increased risk for disease later in the life of the offspring (130).

Table 6: Summary of Placental Markers of growth

Placental Marker	What it indicates
Smaller and Larger disk diameters	Surface area available for spiral arteries
Ratio of the diameters	reflects the asymmetry of placental growth
Disk thickness	Area available for nutrient transfer

1.2.7 Summary of the interrelationship among preeclampsia, fetal sex, fetal growth and placental growth.

The risk of FGR is greater among offspring of preeclamptic women compared to offspring of normotensive women (22). Preterm preeclampsia is more severe than term preeclampsia, it is associated with a greater maternal and fetal morbidity (40). The fact that males are more common in preterm normotensive pregnancies than in preterm preeclamptic pregnancies (106, 108), may indicate increased stillbirths among preterm male offspring of preeclamptic women. In preeclamptic pregnancies there is evidence that that male sex affects vasodilation in both the mother (109) and the fetus (110), also pregnant women with male fetuses have increased blood pressure and weight gain (111). These indicate that male sex has the potential to influence the effect of preeclampsia on fetal development. Male offspring of preeclamptic women tend to fare worse in perinatal outcomes (11, 100, 102). Based on these observations, which state that males are already disadvantaged in pregnancy and can influence the outcome of preeclampsia, we expect to find results consistent with reduced fetal and placental growth among preterm male

offspring of preeclamptic women that will be more severe than similar changes in female fetuses. Several studies that have investigated sex differences in preeclampsia were limited to SGA infants. Our study intends to analyze the population in the CPP data to characterize growth in the offspring and placenta of the preeclamptic versus the normotensive population, and will not be limited to SGA infants. Also, very few studies have investigated the influence of sex on placental morphometry. Our study will address the limitations of SGA while determining the influence of sex on the relationship between preeclampsia and growth restriction in the fetus and the placenta.

1.2.7.1 Gaps in our current knowledge.

Gaps in our current knowledge of the role that fetal sex plays in fetal growth, the growth of the placenta and preeclampsia will begin to be filled by our proposed study. Our literature review revealed limited information describing normal growth using FPR, PI and HCC by race and sex. We intend to fill the gap in this knowledge by using the large cohort of the CPP to characterize normal anthropometric growth among offspring of normotensive women. The alternative markers used in our study will confirm SGA (for fetal growth) and provide more information about reduced fetal/placental growth in pregnancy. Using a racially diverse population, our proposed study will also allow us to describe the contribution of fetal sex to fetal and placental growth in the presence of preeclampsia.

The compensatory mechanisms of the placenta (4), the fetal origins of adult disease hypothesis (130), and the limitations of SGA and FPR, led us to theorize that even though an infant may be characterized at birth as normally grown, a fetus or placenta with reduced growth indicated by our alternative indicators of growth (fetal: ponderal index, head to chest

circumference); (placental: placental weight, smaller diameter, larger diameter, and disk thickness) may reveal growth reductions by sex that may be associated with early and/or later life consequences.

Fetal sex differences that influence placental, fetal and maternal physiology may bring us closer to understanding the pathophysiology of preeclampsia as well as explain why morbidity and mortality rates differ by sex in pregnancy complications. Fetal sex differences may be beneficial in directing future research to interpret the relationship between fetal growth, placental structure/function and the pathophysiology of preeclampsia

1.3 BIOLOGICAL VS. STATISTICAL INTERACTION

Our study aims to determine if the relationship between preeclampsia and fetal growth is modified by infant sex. We will use statistical interaction to detect this biological interaction. Biological interaction implies that two or more risk factors of a disease together influence disease risk. Statistical interactions are used to as a proxy to measure/detect the presence of biological interaction. Statistical interaction refers to a product term in a regression model that detects if the relationship between two variables varies as a function of a third variable (181). Statistical interaction can be measured on an additive or multiplicative scale (182, 183).

True biological interaction indicates a biological pathway or causal mechanism. Rothman illustrates that cases of exposure to two risk factors can be divided into four potential categories of causal mechanisms. Biological interaction can then be estimated from the additivity of risk differences or risk ratios of these categories(184). Therefore it has been argued that measuring

statistical interaction in the additive scale is a better predictor of biological interaction because the additive scale measures synergism of effects(185).

Synergism or positive interaction occurs when the sum of the individual effects is greater than the combined effect (186). This indicates that individuals with both exposures have an increased risk for the outcome, beyond the risk expected from the sum of the exposures. Antagonism or negative interaction occurs when the sum of the individual effects is less than the combined effect (186). This indicates that individuals with both exposures have a decreased risk for the outcome, below the risk expected from the sum of the exposures. The synergy index (SI) can be used to measure departure from additivity of effects in multiplicative models. The SI is calculated as the ratio between the combined effect and individual effects(187). We used the synergy index to estimate the effect of interactions in the additive scale from our SGA models (multiplicative models). SI ranges from 0 to infinity; the SI equals 1 if there is no interaction (exactly additive), values greater than 1 indicate synergism, while values less than 1 indicate antagonism (188).

We will use linear regression models and the synergy index (for the multiplicative models) to detect biological interaction. We will use separate regression models for each marker of fetal and placental growth to evaluate whether fetal and placental growth are modified by infant sex. Multiple fetal and placental markers will enable us to determine whether our findings are consistent and reliable. The results will be reported separately for each sex to interpret the influence of infant sex.

2.0 MANUSCRIPT 1: MODIFYING INFLUENCE OF SEX AND RACE ON DETERMINANTS OF FETAL GROWTH

Manuscript in preparation

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2.1 ABSTRACT

It is unknown if fetal sex and race modify the impact of maternal pre-pregnancy body mass index (BMI), smoking and socio-economic status (SES) on fetal growth. We studied markers of growth in offspring of 8,801 primiparous, normotensive women with singleton pregnancies, enrolled in the Collaborative Perinatal Project. We tested for departures from additivity between sex/race and each determinant. The head-to-chest circumference ratio (HCC) decreased more, while birthweight and ponderal index (PI) increased more for each 1kg/m^2 increase in pre-pregnancy BMI among term females vs. males ($p=0.07$, $p<0.01$ and $p=0.08$, interaction respectively). For term offspring of White compared with Black women, smoking independent of "dose" was associated with larger reductions in growth (165g vs. 68g reduction in birthweight), greater reduction in the fetal placental ratio ($p<0.01$, interaction), PI ($p<0.01$, interaction), and greater increase in HCC ($p=0.02$), respectively). The relationship between SES and fetal growth was not modified by fetal sex. The association of BMI and smoking with fetal growth appeared to be reversed in term compared to preterm infants. Our study provides evidence that the associations of pre-pregnancy BMI and smoking are not constant across fetal sex and race. This may be relevant to sex and race differences in neonatal and long term health outcomes.

2.2 INTRODUCTION

Male infants are significantly heavier and have larger head circumferences at birth compared to female infants (4, 189, 190). Nonetheless males have lower Apgar scores and higher rates of fetal distress (11), are more likely to need resuscitation, have higher rates of perinatal mortality (91) and higher rates of later life disease (191, 192). In addition, infants born to Black mothers are smaller and have higher rates of growth restriction as well as preterm delivery compared to infants born to White mothers (59-62).

Social, behavioral and environmental factors impact fetal growth (193-196). Pre-pregnancy body mass index (BMI) is a useful indicator for evaluating maternal nutritional status near conception (197) and is a predictor of infant birthweight(197, 198). Socio-economic status (SES) is an index of maternal resources to support fetal growth (115, 195), while maternal smoking is an environmental factor that impairs fetal growth(199-201). However little is known about whether maternal pre-pregnancy BMI, SES and smoking interact with maternal race and infant sex to impact of fetal growth. To improve our understanding of factors affecting fetal growth we designed a study to evaluate whether the impact of maternal BMI, smoking and SES on fetal growth are modified by fetal sex and maternal race.

Appropriate growth is essential for both immediate and long term health of the infant. Low birthweight is associated with both acute and later life mortality and morbidity for the infant (4-8). Small for gestational age (SGA) is the most common marker used to detect abnormal growth. It is commonly defined as birthweights below the 10th percentile for babies born at the

same gestational age in a population. An SGA infant however, may not be growth restricted, e.g. small individuals have small babies. Growth restricted infants genetically destined to be large; who do not exercise their true genetic growth potential may not be small enough to be SGA. To address these limitations, additional anthropometric indicators of growth such as ponderal index, head to chest circumference ratio and fetal placental ratio may allow better characterization of impaired fetal growth.

In addition to SGA, our study analyzed these markers of fetal growth among infants born to primiparous normotensive women. The study was designed to evaluate whether the impact of maternal BMI, smoking and SES on fetal growth are modified by fetal sex and maternal race. This may bring us closer to understanding why female and White offspring have a greater resilience and male and Black offspring are at a disadvantage in pregnancy outcomes and long term health consequences.

2.3 METHODS

2.3.1 Study population

The subjects were pregnant women and their offspring who were enrolled in the Collaborative Perinatal Project (CPP), a prospective study of neurologic disorders and other conditions in children. The CPP collected data on 54,681 births to pregnant women enrolled at 12 study centers during 1959 to 1965, about half of whom were African American (202, 203).

Of the 54,681 births in the Collaborative Perinatal Project, 16,523 were primiparous with singleton births (Figure 3). Multiparity is associated with increased birth weight in subsequent pregnancies(204). The risk for adverse pregnancy outcomes also changes with increasing parity (205). Limiting the study to primiparous women removed these confounding effects of parity. We excluded 750 women diagnosed with preeclampsia, 3,425 women diagnosed with proteinuria only, or chronic or transient hypertension (gestational hypertension without proteinuria (206)), 103 that were missing hypertension status and 179 women with diabetes mellitus. These women were excluded to examine the relationship between the predictors of fetal growth and their interaction with sex/race among women that were otherwise healthy. We also excluded stillbirths (n=177), those missing the covariates of interest (n=1,167), Hispanics and Asians (n=706) as well as births less than 25 or greater than 42 weeks or those missing gestational ages (n=1,215). Hispanics and Asians were excluded because of small numbers. The final population was 8,801 normotensive women and their offspring.

Gestational age was calculated based on the last menstrual period of the mother. This method is known to be subject to error (207-209). Women with long cycles are more likely to have their gestation length overestimated and misclassified as term (210) while women with short cycles are more likely to have their gestation length underestimated and misclassified as preterm. To address this limitation, gestational age was defined as reliable in a subgroup of women (n=4,905) with a last menstrual period that was 26 to 35 days following the beginning of the previous menstrual period (211, 212). Analyses were repeated in this group. To further determine if possible bias introduced by misclassification of preterm/term infants affected our results, we performed a second analysis among infants with gestational ages 39-42 weeks. The results were unaffected when we performed both secondary analyses. Therefore the results reported are for the full study sample.

2.3.2 Fetal growth Variables

Birthweights(213), birth lengths (213), head (214, 215) and chest circumferences (215) were checked for plausibility using comparative reference populations. The models met the assumptions for normality after Tukey's severe outlier criterion was used to remove biologically implausible data (216). Among the term infants, 1-19 observations were removed from each growth measure. Among the preterm infants, 0 to 8 outliers were removed from each growth measure. The results of the fetal growth models were not different with the removal of outliers.

The fetal growth variables were SGA, head to chest circumference ratio, ponderal index and fetal placental ratio. SGA was defined as birthweight below the 10th percentile for gestational age and sex from the CPP population. Ponderal index, (100 x [birthweight (g)/crown-heel length $(cm)^3$] is similar to BMI in adults. It indicates those infants who attained insufficient

(low ponderal index) or excess soft tissue growth (high ponderal index) relative to the skeletal growth. Head to chest circumference ratio (HCC), (head circumference (cm) / chest circumference (cm)), indicates compensatory growth resulting in a higher head to chest circumference ratio (148). The fetal placental ratio (FPR) (birthweight (g) / placental weight (g)) is often used as a measure of the efficiency of fetal growth relative to placental growth (27). Growth restricted infants tend to have lower fetal placental ratios (112, 161). In cases where fetal weight appears appropriate for gestational age, these measures may indicate impaired growth in the absence of SGA conversely they may also indicate normal growth in SGA infants. Our study population had large numbers of missing placental weight and chest circumference measures. Women missing placental data (n=1,101, 14.5%) were more likely to be of lower SES, Black and non-smokers, while mothers of offspring missing chest circumference data were more likely to be of higher SES, and White.

2.3.3 Sociodemographic variables

Maternal smoking, SES and race were collected via self-report at the initial interview. BMI was calculated from measured height and self-reported pre-pregnancy weight at admission to the prenatal clinic. Maternal smoking status at intake visit was coded as a yes/no dichotomous variable. The number of cigarettes smoked per day at intake visit was also analyzed as a continuous variable using 5 cigarette increments. The socioeconomic status variable was a combined score based upon education, occupation and income (202, 203) and was categorized as low, middle and high SES. Maternal pre-pregnancy BMI and gestational age were analyzed as continuous variables. Gestational weight gain may be a mediator on the biological pathway between maternal pre-pregnancy BMI and fetal growth. To measure the total influence of BMI

on fetal growth, it is important not to block any effect that acts through a mediating variable (217), therefore we did not control for gestational weight gain.

2.3.4 Statistical analysis

Chi-square tests were used to test for association between variables. Separate multivariable linear regression models were used to evaluate the influence of maternal pre-pregnancy BMI, smoking and SES on fetal growth. Normality and linearity assumptions were tested using histograms of the residuals, q-q plots and residuals vs. fitted values for each model and outliers removed as previously described.

Separate linear and logistic regression models were fitted by sex and race to show the estimate of the relationship between each determinant and fetal growth. Growth restriction early in utero is typically symmetric while growth restriction later in utero tends to be asymmetric(126). In addition, the risk factors for growth restriction differ among preterm and term infants(218). Therefore we analyzed the influence of maternal pre-pregnancy BMI, smoking and SES on fetal growth separately in preterm and term infants. The results were reported as point estimates of the mean and standard error or estimated odds ratio and 95% confidence interval.

Statistical interaction terms between sex and each determinant as well as race and each determinant were used to test if the influence of maternal pre-pregnancy BMI, smoking and SES varied by sex or race. Models with and without interaction terms were compared using the partial F test for linear models and the likelihood ratio test for logistic models ($P<0.10$). It is proposed that departures from additivity (interaction in the additive scale) are more meaningful for interpreting the public health and biological significance than departures from

multiplicativity (interaction on the multiplicative scale) (185, 219, 220). Therefore departures from additive effects in the SGA models were also evaluated by calculating the synergy index (SI) (a test of interaction) (221) and its 95% confidence interval (222). The SI was calculated as the ratio between the combined effect and the sum of the individual effects of the risk factors (187).

The synergy index was originally designed to measure risk factors rather than preventive factors(187). Therefore, we modeled maternal pre-pregnancy BMI with a 1 unit decrease using the World Health Organization categories (Obese (>30kg/m^2) (referent), Overweight (25.0- 29.9 kg/m^2), Normal (18.5-24.9 kg/m^2) and Underweight (<18.5 kg/m^2).

Stata Software, version 11 (StataCorp, College Station, TX) was used to calculate departures from additivity for the SGA logistic regression models. All other statistical analyses were performed using SAS 9.2 software (SAS Institute, Cary, N.C.). A *P* value of <0.05 was considered statistically significant. We repeated the analysis in the preterm and term infants as well as in infants born to women who had reliable menstrual cycles to investigate if the results were consistent.

2.4 RESULTS

Table 7 shows the characteristics of the normotensive women stratified by maternal race and infant sex. Among the births, 1,219 (14%) of the infants were preterm (25 to 36 weeks gestation). There were no meaningful differences in maternal race, smoking status, SES or rates of preterm birth by offspring sex. White mothers were older and smoked more compared to Black mothers (*P*<0.01). Black mothers had more preterm infants, lower SES and were significantly more overweight and obese compared to White mothers (all *P*<0.01).

As expected, the mean birthweight, head to chest circumference, and fetal placental ratios were all higher among male compared to female offspring (P<0.01) (Table 8). Among offspring of Black compared to White women, mean birthweight and ponderal index were lower (P<0.01), while the mean head to chest circumference ratio was higher (P<0.01). Males compared to females had an 18% greater risk of SGA, while Blacks compared to Whites had a 78% greater risk of SGA (males vs. females OR 1.18 (95% CI: 1.02, 1.36) p=0.02 and Blacks vs. Whites OR 1.78 (95% CI: 1.51, 2.10) P<0.01 respectively) using non-race adjusted charts. Importantly, when we compared the risk of SGA in Black and White infants using race adjusted charts(141, 142), SGA rates were similar in Blacks and Whites despite the other markers of reduced growth we found in Black infants.

2.4.1 Fetal sex, Maternal Pre-pregnancy BMI and fetal growth

The influence of pre-pregnancy BMI on fetal growth was modified by fetal sex. Growth among term female infants was greater with increasing maternal pre-pregnancy BMI than that of term male infants (Table 9). The head to chest circumference ratio decreased more and the ponderal index increased more in term females compared to term males for each 1kg/m^2 in maternal pre-pregnancy BMI (P=0.07 and P=0.08, additive interaction respectively). This was driven by a significantly larger increase in the chest circumference (P<0.01, additive interaction) and birthweight (P<0.01, additive interaction) for each 1kg/m^2 increase in the maternal pre-pregnancy BMI for females compared to males. For the SGA models we used the World Health Organization categories (Underweight (<18.5 kg/m^2), normal (18.5-24.9 kg/m^2), overweight (25.0- 29.9 kg/m^2) and obese (>30kg/m^2).We found an increase in the risk of SGA for females but not males for each 1 category decrease in the mother's pre-pregnancy BMI (OR 1.15 (95%CI; 0.94, 1.41 vs. OR 1.76 (95% CI; 1.41, 2.21) male vs. female infants respectively).

Consistent with the additive models, the synergy index (synergy index 0.55 (95% CI 0.30, 0.79)) indicated that males were less sensitive to the influence of maternal pre-pregnancy BMI on SGA risk.

We examined the same relationships among preterm births. Precision was compromised by the smaller sample size resulting in changes that were not statistically significant. Nonetheless, the influence of fetal sex and BMI on fetal growth appeared to be opposite that observed in term pregnancies (all $P>0.19$, interaction), with fetal growth being influenced more by increasing maternal BMI in male vs. female fetuses.

2.4.2 Maternal race, smoking and fetal growth

Smoking was associated with a larger reduction in birthweight in term offspring of White compared to Black women (165 vs. 68 g reduction, respectively) (Table 10). There was also a greater reduction in fetal placental ratio ($P<0.01$, additive interaction), a larger increase in the head to chest circumference ratio ($P=0.02$, additive interaction) and a reduction in the ponderal index ($P<0.01$, interaction) in the term offspring of White compared to Black smokers. Additionally, offspring of White smokers had a higher relative risk of being SGA compared to non-smokers than the offspring of Black smokers in the multiplicative scale (OR 2.90 [95% CI: 2.24, 3.77] versus OR 1.51 [95% CI: 1.24, 1.85]; $P<0.01$ for multiplicative interaction). The borderline significance of the synergy index (synergy index 0.86 (95%CI 0.67, 1.05) was consistent with the additive models which indicated that offspring of Black women were less susceptible to the influence of maternal smoking.

White women tended to smoke more heavily than Black women (7.8 compared to 4.0 cigarettes per day respectively; $P<0.01$). Even after accounting for smoking 'dose' there was still

a greater reduction in birthweight, crown-heel length, and fetal placental ratio and a greater increase in the risk of SGA among offspring of White smokers compared to Black smokers. For each five additional cigarettes smoked per day, the decrease in fetal growth was greater in offspring of White smokers (birthweight, crown heel length, head circumference, chest circumference and fetal placental ratio all $P \leq 0.05$, interaction) (Table 11). When the preterm births were analyzed, the results again appeared to be opposite to that observed among the term infants. With smoking there appeared to be a larger reduction in birthweight in preterm offspring of Black smokers than in preterm offspring of White smokers, although the confidence intervals of these estimates were wide.

Among term infants, socioeconomic status did not significantly impact fetal growth in the linear regression models for fetal placental ratio, head to circumference ratio (P=0.29, 0.17 respectively) but was a significant influence on fetal growth as indicated by the prevalence of SGA ($p<0.001$) and ponderal index (P=0.02) models. The risk of SGA was lower among the highest socioeconomic group compared to the lowest and middle SES groups. In addition SGA risk was lowest among the White vs. Black of the highest SES group.The middle SES group, had a lower mean ponderal index, compared to the lowest SES group. However there was no difference in the mean ponderal index between the highest vs. lowest SES group. Fetal sex or race did not modify the influence of socioeconomic status on ponderal index (data not shown).

2.5 DISCUSSION

In this study we identified pre-pregnancy BMI and maternal smoking as factors that influence fetal growth differently according to maternal race or infant sex. Our findings indicated greater

growth of term female vs. male infants with increasing maternal pre-pregnancy BMI. In addition, the growth of term offspring of white smokers was lower compared to term offspring of Black smokers. Although the precision of our results was compromised by smaller sample size with preterm infants, we observed an opposite relation with increasing maternal pre-pregnancy BMI on fetal growth for preterm males compared to females. Also the direction of the influence of smoking with preterm births was opposite that in term births. Maternal smoking had a larger negative influence among preterm offspring of Black compared to White smokers. These results suggest that maternal pre-pregnancy BMI and smoking influence fetal growth through different biological pathways at different gestations by sex and race.

A recent study performed in Chile supports our findings that fetal growth is sex-specific and time sensitive in response to maternal anthropometry (223). Lampl reported that birthweight in 1,814 male vs. 1,681 female fetuses varied in sensitivity to different maternal weight and height combinations at different gestations. Specifically, male fetuses were sensitive to maternal height of thin mothers in early pregnancy and maternal weight of short mothers in later gestation. Female infants were sensitive to maternal weight of tall mothers early in pregnancy and maternal height of thin mothers later in gestation (223). In addition, the greater fetal growth and decrease in the incidence of SGA with increasing maternal pre-pregnancy BMI among term females compared to male offspring in our study is consistent with a theory proposed by Eriksson. This theory suggests that females respond to the mother's lifetime nutrition and metabolism while males respond to her current nutrition(103). This was demonstrated in the Dutch Famine in which there was greater increased mortality among adult males than females who were in utero during the famine (224). Animal studies also indicate a greater effect of maternal malnutrition on male than female rats(138, 225). The biological mechanisms that may explain these results

remain unknown, however gene (94, 95) and steroidal pathways (96, 97) have been shown to influence birth outcomes differently by fetal sex.

In our study, term offspring of White smokers had greater reductions in birthweight and a greater likelihood of being SGA than term offspring of Black smokers. This is consistent with a prospective cohort study of 925 term Black and White singleton infants (226) and a population study of US births in 1995 to 485,905 Black and 681,600 White mothers(199). Both studies found greater reductions in growth measurements of White offspring compared to Black offspring of smokers (226). In addition, a case-control study of 407 White mothers and 537 Black mothers found a higher risk of SGA among the offspring of White compared to Black smokers as smoking level (200). A few studies have reported that maternal smoking had a greater negative impact on offspring birthweight of Black compared to White women (201, 227-229). These studies were limited to a specific birthweight cut-point (birthweight less than 2500g) and did not address smoking "dose".

The differential impact of smoking on fetal growth may be explained by innate factors associated with race. As an example, tobacco intermediates are detoxified for excretion by enzymes such as the glutathione S-transferase family. A recent study reported that among smokers, those with offspring with the GSTT1(del) genotype had a larger mean reduction in birthweight than infants without the gene deletion (230). Another study found that the a combination of the GSTM1(del) and the GSTT1(del) genotypes intensified the influence of maternal exposure to environmental tobacco smoke on birth weight more than the presence of either genotype alone(231). The combination of the GSTM 1(del) and GSTT1 (del) genotypes as well as the GSTM1 (del) genotype is more common among US Whites (232), while the GSTT1 (del) genotype is more common in US Blacks. More research is needed to determine if

there is a race-gene-smoking interaction which influences fetal growth by race among offspring of smokers with these genotypes.

Our study had several strengths. The Collaborative Perinatal Project (CPP) is the only study of this scale with fetal measurements in the United States. In the CPP, anthropometric data were recorded at birth and were measured using standardized protocols which minimized bias. The equal numbers of Black and White women allowed the opportunity to explore the interaction between race and smoking on fetal growth in a large number of infants. The consistency in the results of reduced growth using several indicators of fetal growth, in addition to SGA, supports our findings and indicates true changes in growth that are unlikely due to chance. We found lower anthropometric measures accompanied by greater odds of SGA among offspring of Blacks compared to Whites in our study. Despite the anthropometric measures indicating reduced growth, this different rate of SGA between Black and White infants was not evident using race adjusted growth charts. Our findings are consistent with the large body of evidence that offspring of Black women are at greater risk for fetal growth restriction than offspring of White women (142, 233, 234), and support the use of non-race adjusted growth curves.

The study was limited to one-time measures of fetal growth taken at birth and as a result does not indicate growth throughout pregnancy. Although our determination of gestational age and thus term and preterm gestation was based on the mothers last menstrual period, testing the results in women with known normal menstrual cycles or delivery dates of at least 39 weeks did not change the findings. Despite the fact that obesity is more common today (30% currently compared to 9% in CPP), we believe the results are generalizable to a modern population. It seems unlikely that the biological mechanism between a 1kg/m^2 increase in the maternal pre-pregnancy BMI and fetal growth would change over time.

Women missing placenta weight and chest circumference data differed from women with these measures. The findings from the placenta and chest circumference models were consistent with the other fetal growth models suggesting that any resulting selection bias is minimal. Given the large sample size the bias introduced from missing data should be small. There should be little selection bias in our study as the selection criteria were independent of offspring sex.

Our study is novel in that it provides evidence that the influence of maternal pre-pregnancy BMI and smoking are not constant across fetal sex and maternal race. This evidence suggests that this relationship may operate through different biological pathways by sex and race. These subtle race and sex differences in growth are not of a magnitude to justify revisions of the current clinical management. However, our findings indicate it is important to incorporate sex differences in the study of mechanisms of fetal growth. Additionally our study indicates that in addition to SGA, several other indicators of fetal growth capture reduced fetal growth. Additionally, these measures confirmed that using non-race adjusted weight for gestational age tables are more appropriate than race adjusted tables. Our results may help to explain sex and race differences in neonatal and long term health outcomes. The relationship between maternal BMI, smoking, fetal growth, fetal sex and race is complex and warrants further study.

2.6 Tables and Figures

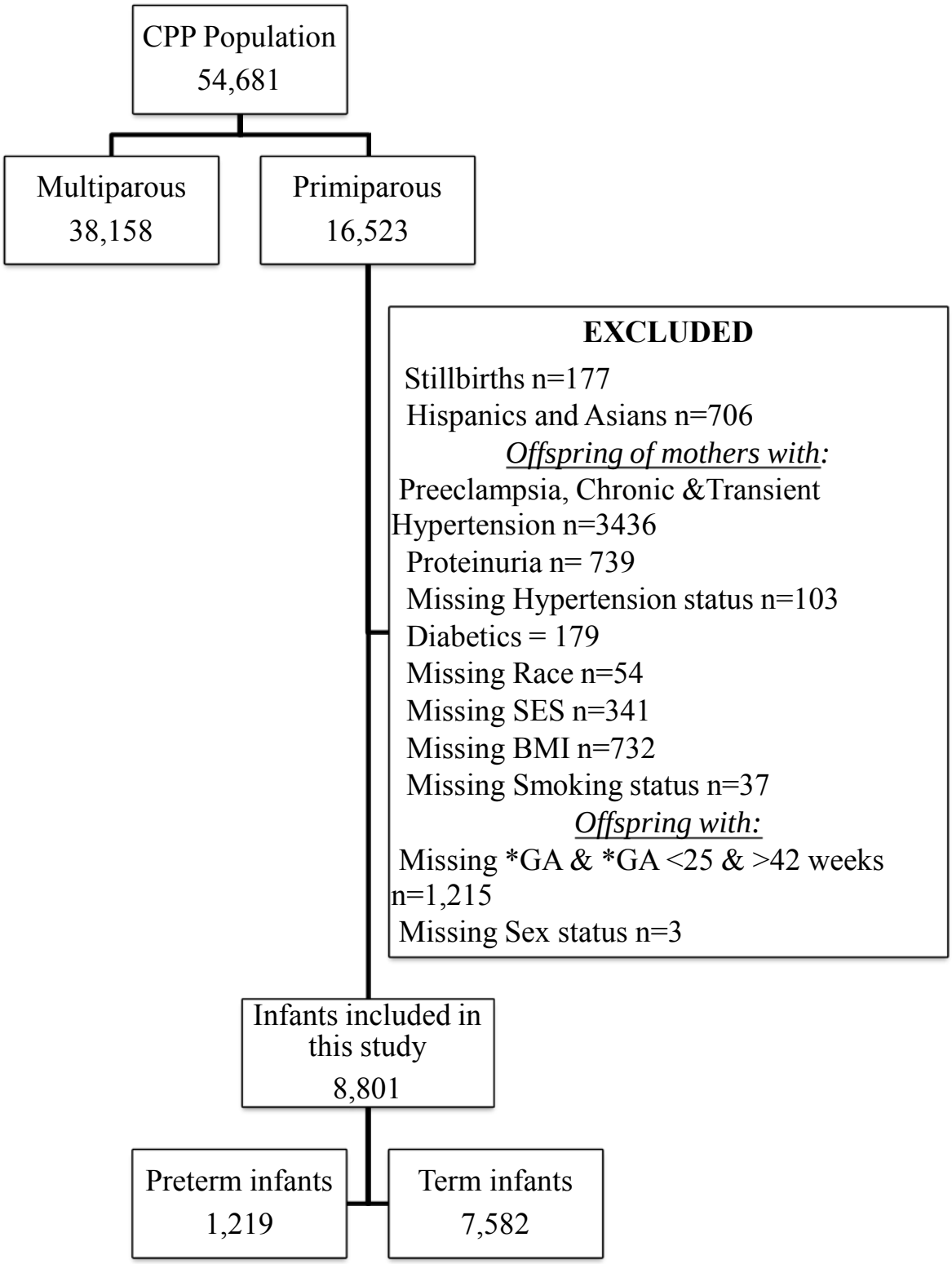

Figure 3: Study Population (Manuscript 1)

Table 7: Baseline Characteristics of Population of Normotensive Primiparous Women

Characteristics	N=8801					
	Males (N=4597) n(%)	Females (N=4204) n(%)	P-value	Blacks (N=4126) n(%)	Whites (N=4776) n(%)	p-value
Maternal Age						
<20	2356 (52.0)	2177 (48.0)	0.22	2800 (61.8)	1733 (38.2)	<0.01
20-29	2125 (52.8)	1898 (47.2)		1218 (30.3)	2805 (69.7)	
30+	116 (47.4)	129 (52.6)		79 (32.2)	166 (67.8)	
Maternal Race						
White	2483 (52.8)	2221 (47.2)	0.27			
Black	2114 (51.6)	1983 (48.4)				
Smoking						
Nonsmoker	2587 (52.5)	2339 (47.5)	0.55	2542 (51.6)	2384 (48.4)	<0.01
Smoker	2010 (51.9)	1865 (48.1)		1555 (40.1)	2320 (59.9)	
Cigarettes/day						
Mean±SD	6.04±12.42	6.17±12.8	0.63	3.99±11.65	7.94±13.09	<0.01
BMI (kg/m^2)						
Underweight (<18.5)	619 (53.8)	532 (46.2)	0.36	569 (49.4)	582 (50.6)	<0.01
Normal (18.5-24.9)	3580 (52.3)	3270 (47.7)		3069 (44.8)	3781 (55.2)	
Overweight (25.0- 29.9)	330 (50.1)	329 (49.9)		374 (56.8)	285 (43.2)	
Obese (>30)	68 (48.2)	73 (51.8)		85 (60.3)	56 (39.7)	
Maternal SES score						
1 (low)	1584 (51.4)	1497 (48.6)	0.49	2275(73.8)	806 (26.2)	<0.01
2 (mid)	1238 (52.4)	1126 (47.6)		1236 (52.3)	1128 (47.7)	
3 (high)	1775 (52.9)	1581 (47.1)		586 (17.5)	2770 (82.5)	
Gestational Age (completed weeks)						
Preterm <37	658 (14.3)	561 (13.3)	0.19	848 (20.7)	371 (7.9)	<0.01
Term ≥ 37	3939 (85.7)	3643 (86.7)		3249 (79.3)	4333 (92.1)	

Table 8: Adjusted Means of the Markers of Growth by Race and Sex for all births (preterm and term)

Fetal Measures	Male [b] Mean (SE)	Female [b] Mean (SE)	Sex Difference	Sex Difference p	Black [c] Mean (SE)	White [c] Mean (SE	Race Difference	Race Difference p
Birthweight (g)	3147.71 (6.53)	3047.38 (6.84)	+100.33	<0.01	3009.74 (7.65)	3178.28 (7.05)	-168.54	<0.01
Crown Heel Length (cm) [a]	50.04 (0.04)	49.38 (0.04)	+0.66	<0.01	49.42 (0.04)	50.00 (0.04)	-0.58	<0.01
Head Circumference (cm) [a]	33.85 (0.02)	33.28 (0.02)	+0.57	<0.01	33.39 (0.02)	33.74 (0.02)	-0.35	<0.01
Chest Circumference (cm) [a]	31.71 (0.03)	31.40 (0.03)	+0.31	<0.01	31.26 (0.03)	31.87 (0.03)	-0.61	<0.01
Placental Weight (g) [a]	426.12 (1.37)	421.57 (1.44)	+4.55	0.02	414.89 (1.67)	430.78 (1.42)	-15.88	<0.01
Ponderal Index [a]	2.507 (0.004)	2.527 (0.005)	-0.020	<0.01	2.493 (0.005)	2.537 (0.005)	-0.044	<0.01
Head- Chest Circumference Ratio [a]	1.070 (0.001)	1.062 (0.001)	+0.008	<0.01	1.070 (0.001)	1.061 (0.001)	+0.009	<0.01
Fetal Placental Weight Ratio [a]	7.57 (0.02)	7.43 (0.02)	+0.14	<0.01	7.48 (0.03)	7.52 (0.02)	-0.04	0.36
SGA n (%)	492 (10.7)	394 (9.4)	-	-	530 (12.9)	356 (7.6)	-	-
SGA OR (95% CI)	1.18 (1.02, 1.36) [d]	REFERENT [d]	-	0.02	1.78 (1.51, 2.10) [e]	REFERENT [e]	-	<0.01

Values are means and standard error unless otherwise specified.

[a]Reduced sample size due to missing values (missing=3% length, PI & head circumference, 12% chest circumference & HCC and 15% placental weight & FPR models).

[b]Derived from linear regression models adjusting for maternal age, race, pre-pregnancy body mass index (BMI), smoking, socio-economic status (SES) and gestational age at delivery.

[c]Derived from linear regression models adjusting for infant sex, maternal age, pre-pregnancy BMI, maternal smoking, SES and gestational age at delivery.

[d]Derived from logistic regression models adjusting for maternal age, race, pre-pregnancy BMI, smoking and SES.

[e]Derived from logistic regression models adjusting for infant sex, maternal age, pre-pregnancy BMI, smoking and SES.

Table 9: Influence of 1 kg/m^2 increase in BMI by Fetal sex and Race on each Growth Measure.

Fetal Measures	Term Male [b] Beta coefficient (SE)	Term Female [b] Beta coefficient (SE)	SEX x BMI Interaction p	Term Black [c] Beta coefficient (SE)	Term White [c] Beta coefficient (SE)	RACE x BMI Interaction p
Birthweight (g)	9.83 (2.28)*	20.23 (2.20)*	**<0.01**	15.15 (2.24)*	14.66 (2.23)*	0.78
Crown Heel Length (cm) [a]	0.034 (0.012)*	0.060 (0.012)*	**0.14**	0.061 (0.013)*	0.033 (0.012)*	**0.07**
Head Circumference (cm) [a]	0.024 (0.007)*	0.045 (0.007)*	**0.06**	0.034 (0.008)*	0.035 (0.007)*	0.89
Chest Circumference (cm) [a]	0.032 (0.010)*	0.073 (0.010)*	**<0.01**	0.056 (0.010)*	0.048 (0.010)*	0.63
Placental Weight (g) [a]	2.482 (0.504)*	4.234 (0.509)*	**0.01**	2.865 (0.535)*	3.723 (0.483)*	0.29
Ponderal Index[a]	0.003 (0.002)†	0.008 (0.002)*	**0.08**	0.004 (0.002)†	0.007 (0.002)*	**0.06**
Head- Chest Circumference Ratio[a]	-0.0004 (0.0003)	-0.001 (0.0003)*	**0.07**	-0.0009 (0.0003)*	-0.0005 (0.0003)	0.35
Fetal Placental Weight Ratio[a]	-0.023 (0.007)*	-0.022 (0.007)*	0.92	-0.014 (0.008)	-0.029 (0.007)*	0.16
	Male [d] OR (95% CI)	**Female [d] OR (95% CI**	**P Interaction**	**Black [e] OR (95% CI)**	**White [e] OR (95% CI)**	**P Interaction**
SGA (<10th percentile) Decrease in BMI WHO[f] category	1.15 (0. 94, 1.41)	1.76 (1.41, 2.21)*	**<0.01**[h]	1.46 (1.21, 1.77)	1.27(1.00, 1.62)	0.33 [h]
SI (decrease in BMI WHO[f] categories from obese to underweight) [g]	**0.55(95% CI; 0.30, 0.79)**		Risk factors= decreasing BMI and Male sex	1.49 (95% CI; 0.55, 2.42)		Risk factors= Decreasing BMI and Black race

*=p≤0.01, †=p≤0.05 The p-values reported are for interaction in additive scale unless otherwise specified

[a]Reduced sample size due to missing values (missing=3% length, PI & head circumference, 12% chest circumference & HCC and 15% placental weight & FPR models).

[b]Derived from linear regression models adjusting for maternal race, age, smoking, SES and infant gestational age at delivery.

[c]Derived from linear regression models adjusting for infant sex and gestational age at delivery and maternal smoking, age and SES.

[d]Derived from logistic regression models adjusting for maternal race, age, socioeconomic status (SES), and smoking.

[e] Derived from logistic regression models adjusting for infant sex infant and maternal age, smoking and socioeconomic status (SES).

[f]World Health Organization BMI Categories Underweight (<18.5 kg/m^2), Normal (18.5-24.9 kg/m^2) Overweight (25.0- 29.9 kg/m^2) Obese (>30kg/m^2)

[g] A synergy index of 1.0 = no interaction, <1 indicates antagonism, and >1 indicates synergy.

[h] p-value for interaction in multiplicative scale

Table 10: Differential Influence of Smoking by race.

Fetal Measures	Non Smoker Mean (SE)	Smoker Mean (SE)		Nonsmoker Mean (SE)	Smoker Mean (SE)		SMOKE x RACE Interaction p
	Term Black[b]		Black difference	Term White[b]		White difference	
Birthweight (g)	3105.87 (9.52)	3037.74 (11.99)	-68.13*	3332.22 (9.13)	3167.32 (8.96)	-164.90*	**<0.01**
Crown Heel Length (cm) [a]	49.92 (0.05)	49.44 (0.07)	-0.48*	50.66 (0.05)	49.98 (0.05)	-0.68*	**0.06**
Head Circumference (cm) [a]	33.72 (0.03)	33.41 (0.04)	-0.31*	34.07 (0.03)	33.75 (0.03)	-0.32*	0.80
Chest Circumference (cm) [a]	31.65 (0.04)	31.34 (0.05)	-0.31*	32.43 (0.04)	31.90 (0.04)	-0.52*	**0.01**
Placental Weight (g) [a]	417.01 (2.28)	422.45 (2.75)	+5.44	434.47 (1.98)	438.09 (1.96)	+3.62	0.68
Ponderal Index[a]	2.497 (0.007)	2.514 (0.008)	+0.017	2.562 (0.006)	2.536 (0.006)	-0.027*	**<0.01**
Head- Chest Circumference Ratio[a]	1.067 (0.001)	1.068 (0.001)	+0.001	1.053 (0.001)	1.060 (0.001)	+0.007*	**0.02**
Fetal Placental Weight Ratio[a]	7.627 (0.032)	7.348 (0.039)	-0.279*	7.836 (0.028)	7.377 (0.028)	-0.459*	**<0.01**
SGA n (%)	279 (13.6%)	238 (19.5%)	-	101 (4.5%)	255 (12.0%)	-	-
SGA (<10th percentile) [c] **OR (95% CI)**	REFERENT	OR 1.51 (1.24, 1.85)*	-	REFERENT	OR 2.90 (2.24, 3.77)*	-	**<0.01**[d]
SI (95% CI) [e]	**0.86 (0.67, 1.05)** Risk factors =Black race and smoking						**0.89**

*= p<0.01, †= p≤0.05. Values are means and standard error unless otherwise specified. P values reported are for interaction in additive scale unless otherwise specified

[a]Reduced sample size due to missing values (missing=3% length, PI & head circumference, 12% chest circumference & HCC and 15% placental weight & FPR models).

[b]Derived from linear regression models adjusting for infant sex infant, gestational age at delivery and maternal age, pre-pregnancy body mass index (BMI) and SES.

[c]Derived from logistic regression models adjusting for infant sex and maternal age, pre-pregnancy body mass index (BMI), and socioeconomic status.

[d] p-value for interaction in multiplicative scale.

[e] A synergy index of 1.0 = no interaction, <1 indicates antagonism, and >1 indicates synergy

Table 11: Influence of a 5 cigarette increase in cigarette consumption per day by Race and Sex on each Growth Measure at term.

Fetal Measures	Term Male [b] Beta coefficient (SE)	Term Female [b] Beta coefficient (SE)	Cigarette x SEX Interaction p	Term Black [c] Beta coefficient (SE)	Term White [c] Beta coefficient (SE)	Cigarette x RACE Interaction p
Birthweight (g)	-13.50 (2.69)*	-11.35 (2.58)*	0.50	-0.59 (2.89)	-19.76 (2.45)*	**<0.01**
Crown Heel Length (cm) [a]	-0.038 (0.015)*	-0.044 (0.014)*	0.82	-0.007 (0.016)	-0.070 (0.013)*	**<0.01**
Head Circumference (cm) [a]	-0.033 (0.009)*	-0.032 (0.008)*	0.80	-0.018 (0.010)	-0.042 (0.007)*	**0.05**
Chest Circumference (cm) [a]	-0.040 (0.012)*	-0.032 (0.012)*	0.49	-0.012 (0.013)	-0.052 (0.011)*	**0.01**
Placental Weight (g) [a]	0.500 (0.573)	0.515 (0.583)	0.79	0.826 (0.647)	0.327 (0.529)	0.54
Ponderal Index [a]	-0.004 (0.002)†	-0.003 (0.002)	0.53	-0.001 (0.002)	-0.004 (0.002)*	0.22
Head- Chest Circumference Ratio [a]	0.0003 (0.0003)	0.00005(0.0003)	0.55	0.0002 (0.0004)	0.0004 (0.0003)	0.20
Fetal Placental Weight Ratio [a]	-0.042 (0.008)*	-0.036 (0.008)*	0.76	-0.013 (0.010)	-0.057 (0.007)*	**<0.01**
	Term Male [d] OR (95% CI)	**Term Female [d] OR (95% CI**	**P Interaction**	**Term Black [e] OR (95% CI)**	**Term White [e] OR (95% CI)**	**P Interaction**
SGA (<10th percentile)	1.07 (1.03, 1.11)*	1.05(1.01, 1.09) †	0.46[n]	1.02(0.98, 1.06)	1.10(1.06, 1.14)*	**<0.01** [f]
SI (95% CI) 5-10 cigs/day [g]	1.12 (0.87, 1.38) Risk factors=male sex & smoking			**0.94 (0.86, 1.01)** Risk factors= Black race & smoking		

*=p≤0.01, †=p≤0.05 The p-values reported are for interaction in additive scale unless otherwise specified

[R]Reduced sample size due to missing values (missing=3% length, PI & head circumference, 12% chest circumference & HCC and 15% placental weight & FPR models).

[b]Derived from linear regression models adjusting for maternal race, pre-pregnancy body mass index (BMI), socioeconomic status and infant gestational age at delivery.

[c]Derived from linear regression models adjusting for maternal pre-pregnancy body mass index and socioeconomic status and infant sex and gestational age at delivery.

[d]Derived from logistic regression models adjusting for maternal race, pre-pregnancy body mass index (BMI), and socioeconomic status (SES).

[e]Derived from logistic regression models adjusting for maternal pre-pregnancy body mass index (BMI), socioeconomic status (SES) and infant sex.

[f] p-value for interaction in multiplicative scale.

[g] A synergy index of 1.0 = no interaction, <1 indicates antagonism, and >1 indicates synergy

3.0 MANUSCRIPT 2: PREECLAMPSIA AND REDUCED FETAL GROWTH: INFLUENCE OF INFANT SEX

Manuscript in preparation

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3.1 ABSTRACT

We investigated whether the relationship between preeclampsia and fetal growth is modified by infant sex. The subjects were offspring of 516 preeclamptic and 8801 normotensive primiparous Black and White women enrolled in the Collaborative Perinatal Project (1959 -1965). The frequency of small for gestational age (SGA<10^{th} centile), mean birthweight, ponderal index, head and chest circumferences were examined. Interaction terms between sex and preeclampsia were evaluated after adjustment for confounders. The results were stratified by preterm status (<37 weeks). Male preterm offspring of preeclamptic mothers had greater reductions in head and chest circumferences than preterm female offspring of preeclamptic women (p=0.02, p=0.01; interaction respectively). The influence of preeclampsia on growth of offspring born at term was more modest, and the influence of sex was opposite that in preterm infants. Compared to term offspring of normotensive women, the reduction in mean ponderal index was greater for female vs. term male offspring of preeclamptic women (p<0.01, interaction). Fetal growth was more impaired among male vs. female preterm infants born to preeclamptic women. This suggests that preterm males born to preeclamptic women may be more prone to neonatal complications. Sex differences in fetal growth may help explain sex differences in neonatal and long term health outcomes.

3.2 INTRODUCTION

Preeclampsia is a pregnancy specific disease that is diagnosed by new-onset proteinuria and increased blood pressure. It affects 3-5% of pregnancies (36), and is associated with abnormal remodeling of the maternal vessels supplying the placenta and reduced fetal and placental growth. The infant faces detrimental effects due to reduced nutrient and oxygen transfer during fetal development.

The effect of preeclampsia on the mother has been well studied. However, there is less information on the specific effects of preeclampsia on offspring. It is evident that male fetuses are at a disadvantage in pregnancy. Spontaneous preterm labor (9) and complications of pregnancies such as fetal distress during labor and low Apgar scores are more common with pregnancies bearing male compared to female fetuses (10, 11). Guided by evidence of sex differences in several pregnancy outcomes, we examined whether the relationship between preeclampsia and fetal growth is modified by infant sex.

Fetal growth restriction (FGR) is defined as fetal growth that fails to achieve the genetic growth potential. Small for gestational age (SGA) is used to detect FGR. It is commonly defined as birthweights below the 10th percentile for babies born at the same gestational age in an appropriate reference population. However, not all SGA infants are small due to impaired growth (e.g. small parents have appropriately grown small infants) and not all FGR infants are identified using SGA criteria. Infants may fall within the normal range of birth weight but show metabolic, hematologic, and neurologic characteristics as seen in growth-restricted infants (235).

For this reason we related preeclampsia and fetal sex to several other indicators of fetal growth: Fetal placental ratio, ponderal index and head to chest circumference ratio. Our study analyzed these markers of fetal growth among infants born to primiparous preeclamptic and normotensive women. The study was designed to evaluate whether fetal growth in preeclamptic offspring is modified by infant sex. Fetal sex differences that influence placental, fetal and maternal physiology may bring us closer to understanding the pathophysiology of preeclampsia as well as provide insight into why morbidity and mortality rates differ by sex in pregnancy and in adulthood.

3.3 METHODS

3.3.1 Study Population

The subjects were pregnant women and their offspring who were enrolled in the Collaborative Perinatal Project (CPP), a prospective study of neurologic disorders and other conditions in children. The CPP collected data on 58,806 pregnant women enrolled at 12 study centers during 1959 to 1965, about half of whom were African American (202, 203).

The present analysis was limited to primiparous Black and White women with singleton births for several reasons. There are pathological and epidemiological findings indicating differences between preeclampsia when it occurs in first or later pregnancies (236). Primiparity is also associated with increased risks of preeclampsia and preterm birth. Limiting the study to primiparous women provided a greater proportion of women with these events. Hispanics and Asians were excluded because of small numbers (7.0%, n= 796) (Figure 4). Women were excluded if they had proteinuria only (n=739), chronic or transient hypertension of pregnancy

(gestational hypertension without proteinuria (206)) (n=2,686) or were missing hypertension status (n=103). Gestational age was limited to 25 to 42 weeks because preeclampsia was defined as occurring after 24 weeks and pregnancies longer than 42 weeks may be associated with complications that could confound our study (excluded n=1,313). Births with missing gestational ages (n=46) and sex of the infant (n=18) were excluded. Women missing the covariates of interest were also excluded (n=1,284).

Gestational age was calculated based on the last menstrual period of the mother. This method is known to have error which may result in the misclassification of preterm and term status (207-209). Women with long cycles are more likely to have their gestation overestimated (misclassified as term) (210) while women with short cycles are more likely to have their gestation underestimated (misclassified as preterm). To address this potential limitation, gestational age was defined as reliable in a subgroup of women (n=5951, 63%) with a last menstrual period that was 26 to 35 days following the beginning of the previous menstrual period (211, 212). Analyses were repeated in this group. To further determine if possible bias introduced by misclassification of preterm/term infants affected our results, we performed a second analysis among infants with gestational ages 39-42 weeks. This was compared to the original distribution of term infants (37-42 weeks) to determine if the findings were the same.

Birth weights (213), birth lengths (213), head (214, 215) and chest circumferences (215) were checked for plausibility using comparative reference populations and Tukey's criteria was used to remove extreme outliers(216). The models met the assumptions for normality after removal of the following outliers: Among the term infants, 0-24 outliers were removed from each model. Among the preterm infants, 0 outliers were removed from each model. The results of the term fetal growth models were not different with the removal of outliers and thus outliers

were removed in the analysis reported in this paper. The final study population was 9317 infants (from 516 preeclamptic and 8801 normotensive women). Our final study sample had large numbers of missing placental weight (n=1433, 15.4%) and chest circumference measures (n=1062, 11.4%). Women missing placental data were more likely to be of lower SES, Black and non-smokers, while mothers of offspring missing chest circumference data were more likely to be normotensive, of higher SES, and White. However it appeared that this was driven by missing data from specific study sites and not due to demographic characteristics of the mother (e.g. 70% of placental data was missing among women from Tennessee, while 25% and 28% of women from Boston and Buffalo sites respectively had chest circumference data missing).

3.3.2 Definition of Preeclampsia

Preeclampsia was determined by gestational hypertension and proteinuria, which terminated in the postpartum period. Gestational hypertension was defined as at least 2 measurements of systolic blood pressure >=140 mmHg and/or diastolic blood pressure >=90 mmHg with no prior occurrence before 24 weeks of gestation. Proteinuria was 2 random urine dipsticks of 1+ protein or one dipstick of 2+ protein(237). Preeclampsia was considered preterm with gestational age at delivery ranging from 25 to 36 completed weeks. Term preeclampsia was preeclampsia with gestational age at delivery ranging from 37 to 42 completed weeks.

3.3.3 Fetal Growth Variables

SGA was considered as birth weight < 10th percentile for gestational age, and sex using the CPP population. Head to chest circumference ratio was the head circumference (cm) / chest circumference (cm). Ponderal index was 100 x [birth weight (g)/crown-heel length $(cm)^3$]. The

fetal placental ratio was the birthweight (g) / placental weight (g). In addition to SGA and birth weight, we evaluated ponderal index which is similar to BMI in adults, and provides an estimate of the relative thinness of the infant. It indicates those infants who attained insufficient (low ponderal index) or excess soft tissue growth (high ponderal index) relative to the skeletal growth. The ratio of head circumference to chest circumference indicates compensatory growth. In the presence of impaired growth, visceral organ growth is compromised earlier and brain growth is spared longer resulting in a higher head to chest circumference (148). The ratio of fetal weight to placental weight (FPR) is often used as a measure of the efficiency of fetal growth relative to placental growth (27). Growth restricted infants tend to have lower fetal placental ratios (112, 161). These markers can help confirm that an SGA infant is in fact failing to manifest its genetic growth potential. Furthermore, in cases where fetal weight may appear appropriate for gestational age, these measures may indicate infants with impaired growth. In addition, using the FPR, ponderal index or head chest circumference can overcome some of the limitations of SGA by avoiding the influence of genetically mediated differences in birth weight.

3.3.4 Socio-demographic Variables

Maternal smoking, socio-economic status (SES), and race were collected by self-report; BMI was calculated from maternal height (measured at enrollment) and self-reported pre-pregnancy weight. Maternal smoking during pregnancy was coded as a dichotomous variable (yes/no). The socioeconomic status variable was a combined score based upon education, occupation and income (202) and was categorized as low, middle and high SES. Maternal pre-pregnancy BMI and gestational age were analyzed as continuous variables.

Stillbirths are the most severe outcome following fetal growth restriction. We hypothesized that if the relationship between preeclampsia and fetal growth is sex-specific, then we should find a similar sex-specific relationship with the rates of stillbirth. In addition, previous studies suggest that there is an excess of female infants in preterm preeclamptic pregnancies (106, 108), perhaps due to excess male stillbirths.

3.3.5 Statistical Analysis

Chi-square tests were used to test for association between variables. Interaction terms between sex and preeclamptic status were evaluated to determine if the influence of preeclampsia on fetal growth varied with fetal sex. Significant interactions were evaluated using the partial F test for linear models and the likelihood ratio test for logistic models ($p<0.10$). It has been proposed that departures from additivity (interaction in the additive scale) are more meaningful for interpreting the public health and biological significance than departures from multiplicativity (interaction on the multiplicative scale) (185, 219, 220). Therefore departures from additive effects in the SGA models were evaluated by calculating the synergy index (SI) (a test of interaction) (221) and its 95% confidence interval (222). The SI was calculated as the ratio between the combined effect and the sum of the individual effects of the risk factors (187). The synergy index was designed to measure risk factors rather than preventive factors. Therefore in the analysis of the SI for the multiplicative SGA model for the preterm infants, we remodeled female sex as the risk factor.

Separate linear and logistic regression models were then fitted for males and females to report the estimate of the relationship between preeclampsia and fetal growth by sex. The results were stratified by preterm status because the influence of preeclampsia is more severe among preterm infants (40, 41). The results were reported as point estimates of the mean and standard

error or estimated odds ratio (OR) and 95% confidence interval (CI). A p value of <0.05 was considered statistically significant. We repeated all analysis in the preterm and term births infants born to women who had reliable menstrual cycles as well as among term infants 39-42 weeks to investigate if the results were consistent with the general analyses. The results were unaffected when we performed both secondary analyses. Therefore results are reported for the full study sample. Stata Software, version 11 (StataCorp, College Station, TX) was used to calculate departures from additivity for the SGA logistic regression models. All other statistical analyses were performed using SAS 9.2 software (SAS Institute, Cary, N.C.).

3.4 RESULTS

The cohort was primarily young mothers, with 52% being under 20 years of age. Forty seven percent of the women were Black, and the majority of women (78%) were of normal BMI (18.5-24.9kg/m^2) (Table 12). There were 527 preeclamptic births (5.6% of study population. There was a greater percentage of males among the preeclamptic women below 20 years of age but a larger percentage of females among preeclamptic women 20-29 years of age (P<0.01). However, there were no differences in maternal age by sex among the normotensive (P=0.22). There were more smokers among the normotensive women compared to preeclamptic women (P<0.01) and more Black women among the preeclamptic compared to normotensive group (P<0.01). There were more overweight, obese, and low SES women as well as more SGA infants among the preeclamptic compared to normotensive group (all $P\leq$0.02).

The male to female sex ratio of live births was modestly different in the normotensive and preeclamptic groups. Overall, male offspring were more common among normotensive

women but less common among offspring of preeclamptic mothers (male:female sex ratios: overall 1.09 vs. 0.94 for normotensive vs. preeclamptic women respectively, *P*=0.08). As expected, we found that among offspring of preeclamptic women, males were less common in preterm deliveries than at term (male:female sex ratios preterm vs. term: 0.89 vs. 0.94). In contrast, among offspring of normotensive women, males were more common in preterm compared to term births (male:female sex ratios preterm vs. term 1.15 vs. 1.08). In addition, we also investigated if an excess of male stillbirths among offspring of preeclamptic women could explain why males were less common in preterm preeclamptic pregnancies, than among term preeclamptic pregnancies. There were 105 stillbirths (stillbirths among preeclamptic women n=22) (Table 12). We found higher rates of stillbirths among offspring of preeclamptic women than normotensive women (4.10% vs. 0.93%; *P*<0.01). However, the rate of stillbirth did not vary by infant sex (*P*=0.61, and *P*=0.87 for normotensive and preeclamptic women respectively).

3.4.1 Preterm Infants

When compared to preterm offspring of normotensive women, there was a greater reduction in fetal growth among preterm males compared to preterm females born to preeclamptic women (Table 13). When comparing the infants of preeclamptic women with those of normotensive women, there was a greater reduction in the crown-heel length (-1.67 vs. -0.25; P=0.06, additive interaction), head circumference (-1.06cm vs. +0.10cm; P=0.02, additive interaction), chest circumference (-1.61cm vs. -0.05cm; P=0.01, additive interaction) and birthweight (-340 vs. -60g; P=0.05, additive interaction) of preterm males compared to preterm females. The synergy index 0.38 (95% CI 0.00-0.84) was consistent with the linear models and suggested that females are less susceptible than males to exposure to preterm preeclampsia. When the infants born

preterm to women with reliable menstrual cycles only were analyzed, the results were consistent with this preterm analysis (data not shown).

3.4.2 Term Infants

The association between preeclampsia and fetal growth among the term infants was more modest and the relationship with infant sex appeared to be opposite that observed among the preterm infants (Table 14). When compared to term offspring of normotensive women, females had a more significant reduction in the ponderal index compared to male infants of preeclamptic women (0.11 vs. 0.04, respectively; $P<0.01$ additive interaction). The relation of preeclampsia with other growth parameters did not appear to be modified by infant sex.

3.5 DISCUSSION

Our findings indicate that the influence of preeclampsia on fetal growth was modified by fetal sex. These results suggest that preeclampsia may impact fetal growth through different biological pathways by infant sex. Among infants born preterm, decreased growth was more prominent in male vs. female newborn offspring of preeclamptic compared to normotensive women. In contrast, among the term offspring we found a more modest reduction in fetal growth, with females being more affected.

In animal models, studies of insults during fetal development such as dietary under-nutrition (238), hypoxia (239) and placental insufficiency (240, 241) have a greater negative influence on male compared to female animals. In humans studies, low ponderal index (very lean

mass) at birth is associated with a greater risk for cardiovascular death in men compared to women (p=0.01, for sex interaction) (191). In addition, low birth weight (<2500g) is associated with a greater risk for chronic kidney disease among men but not women (p=0.03, sex interaction) (192). There is also a growing body of evidence suggesting that there is a sex-specific fetal response to maternal disease during pregnancy. Our findings are consistent with the majority of studies that indicate a male disadvantage in pregnancy outcome in response to in utero insults. For example in diabetic (98, 99) and chronic hypertensive (100) pregnancies, male infants are at higher risk of morbidity and mortality. Therefore, we speculate that our findings of reduced fetal growth among male relative to female offspring of preeclamptic women may be indicative of greater susceptibility to later life disease.

Our findings of differential relationships between preeclampsia and growth reduction among males vs. females delivered preterm and term is consistent with studies of asthma and fetal growth. Murphy found that among pregnant asthmatic women who did not use inhaled steroid for treatment of asthma, the female fetuses showed greater reductions in growth, while the growth of the males was unaffected (101). However, among pregnant asthmatic women with severe exacerbations that required hospitalization, male infants had greater reductions in birthweight(102). This is consistent with the theories proposed by Clifton(94) and Eriksson(103) that when faced with maternal insults, males maximize continued fetal growth while females reduce growth to increase chances of survival. However when faced with continued or increased in utero insult, males are at greater risk for adverse outcome because they have exhausted their placental reserves (94, 103). This may explain our findings in the preterm and term infants. Early onset of preeclampsia is more likely to be severe (242) and in general more likely to be associated with growth restriction than preeclampsia that occurs near term (160). As a result, this

greater in utero insult among preterm infants may explain the greater reductions in growth among male vs. female offspring.

Among offspring of preeclamptic women, males were less common in preterm deliveries than at term. In contrast, among offspring of normotensive women, males were more common in preterm compared to term births. This is consistent with at least two large studies that also found that male offspring were less common among preterm preeclamptic pregnancies, than among term preeclamptic pregnancies (106, 108). We could not demonstrate an excess of stillbirths by fetal sex that could explain this finding. However, the number of stillbirths to preeclamptic women was small and thus we may not have had sufficient power to detect small sex differences in the rates that may explain the difference in live births.

Our study had several strengths. The Collaborative Perinatal Project (CPP) is a large dataset, which allowed the exploration of the relatively rare adverse pregnancy outcome-preeclampsia. The CPP also included large numbers of fetal measures collected according to standardized research protocols. To our knowledge, this is the only study of this scale with these measurements in the United States. The CPP data allowed us to explore the relationship between preeclampsia and fetal growth in a racially diverse population. The management of preeclampsia has changed since the 1960s, when there were fewer interventions for fetal indications. This allowed us to investigate the natural progression of the disease on fetal growth than would be possible in a modern cohort and thus limits our generalizability. However, we expect that the sex differences observed in the CPP population are generalizable to a modern population. There is little possibility of systematic differences between the biological pathway and management of preeclampsia by infant sex.

Ultrasound technology which is currently used to monitor fetal growth was not available in the 1960s. As a result, the study was limited to measures of fetal measures that were taken at birth and therefore does not indicate growth rate throughout pregnancy. Errors that exist in the measurement of the anthropometric factors may be amplified in the calculation of the head-chest circumference, ponderal index and fetal placental ratio. However there is little possibility of systematic differences in these measures and ratios by preeclamptic status or infant sex.

We repeated the analyses among women whose last menstrual period was 26 to 35 days following the previous menses and among births between 39-42 weeks of gestation to check for misclassification bias. These results were consistent with the term analysis (37-42 weeks), suggesting that potential bias introduced by unreliable gestational ages may be minimal. Our findings indicate that preeclampsia may influence fetal growth differently among term and preterm infants. Preeclampsia may also influence fetal growth differently in very early (births <32 weeks) and late preterm births (32-36 weeks). However, due to small numbers of early preterm infants, our study was unable to investigate the influence of preeclampsia on fetal growth in early vs. late preterm infants.

The consistency in the results of the multiple measures of fetal growth in our study provides reassurance that impaired growth is subtly different in preeclamptic offspring based on sex. This evidence suggests that the relationship between fetal growth and preeclampsia may be influenced through different biological pathways by fetal sex. These growth reductions are not of a magnitude to justify alterations in current clinical treatment. However, the findings from our study indicate the importance of including sex differences in the study of mechanisms of fetal growth. The relationship between preeclampsia, fetal growth and fetal sex is complex and warrants further study.

3.6 Tables and Figures

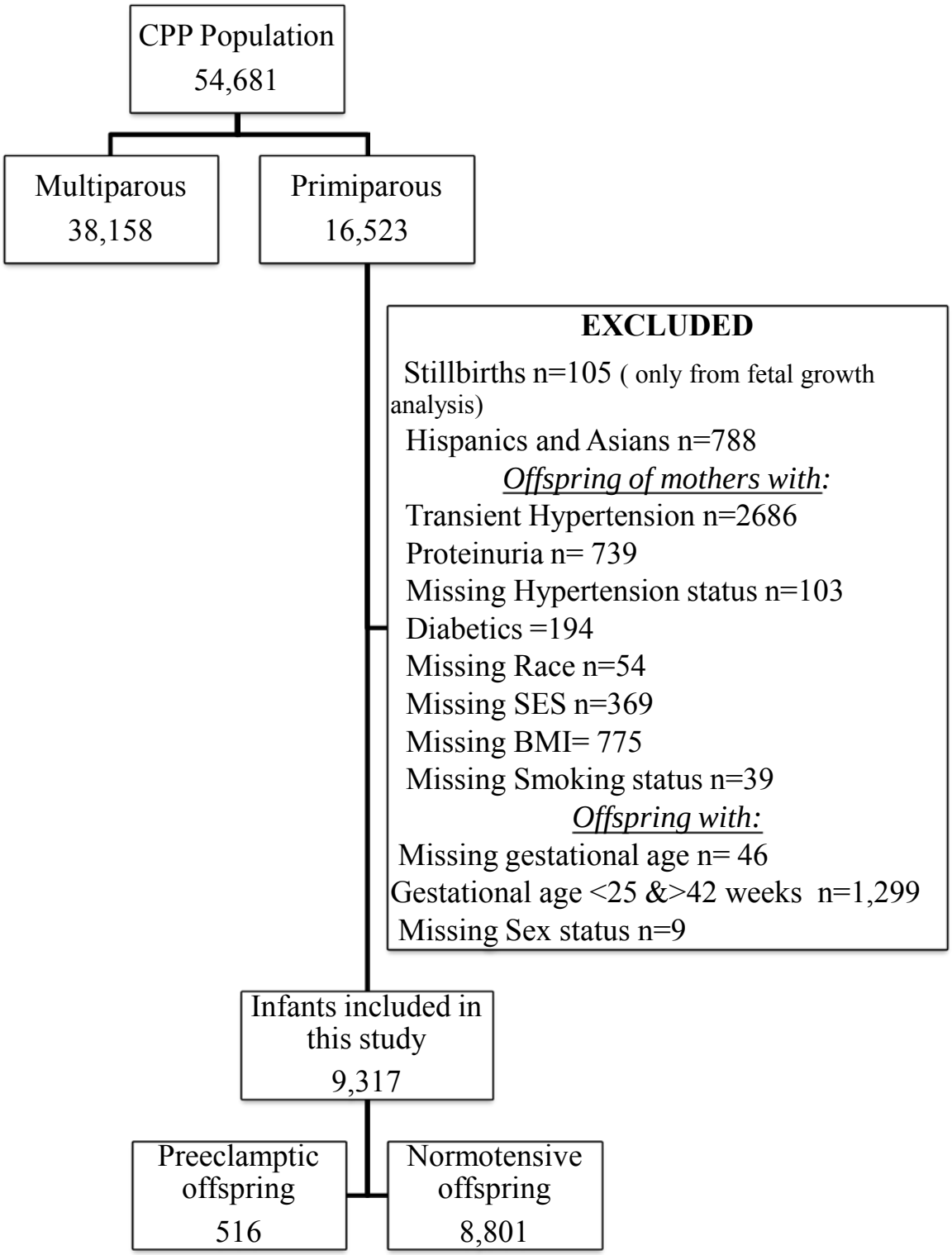

Figure 4: Study Population (Manuscript 2)

Table 12: Baseline Characteristics of Population of Primiparous Women with live births (n=9,317)

Characteristics	Preeclampsia N=516			Normotensive N=8801				PE vs. Ctrl Males	PE vs. Ctrl Females
	Males (N=249) n (%)	Females (N=267) n(%)	P-Value	Males (N=4597) n(%)	Females (N=4204) n(%)	P-value		P-value	P-value
Maternal Age									
<20	165 (66.3%)	164 (61.4%)	0.02	2356 (51.3%)	2177 (51.8%)	0.22		<0.01	<0.01
20-29	76 (30.5%)	102 (38.2%)		2125 (46.2%)	1898 (45.1%)				
30+	8(3.2%)	1 (0.4%)		116 (2.5%)	129 (3.1%)				
Maternal Race									
White	105 (42.2%)	94 (35.2%)	0.10	2483 (54.0%)	2221(52.8%)	0.27		<0.01	<0.01
Black	144 (57.8%)	173 (64.8%)		2114 (46.0%)	1983 (47.2%)				
Smoking									
Nonsmoker	165 (66.3%)	182 (68.2%)	0.65	2587 (56.3%)	2339 (55.6%)	0.55		<0.01	<0.01
Smoker	84 (33.7%)	85 (31.8%)		2010 (43.7%)	1865 (44.4%)				
BMI (kg/m^2)									
Underweight (<18.5)	26 (10.5%)	29 (10.9%)	0.95	619 (13.6%)	532 (12.7%)	0.36		0.01	<0.01
Normal (18.5-24.9)	188 (76.1%)	196 (73.7%)		3580 (78.4%)	3270 (78.1%)				
Overweight (25.0- 29.9)	27 (10.8%)	32 (12.0%)		330 (7.2%)	329 (7.8%)				
Obese (>35)	8 (3.2%)	10 (3.7%)		68 (1.5%)	73 (1.7%)				
Maternal SES score									
1 (low)	124 (49.8%)	142 (53.2%)	0.61	1584 (34.5%)	1497 (35.6%)	0.49		<0.01	<0.01
2 (medium)	70 (28.1%)	65 (24.3%)		1238 (26.9%)	1126 (26.8%)				
3 (high)	55 (22.1%)	60 (22.5%)		1775 (38.6%)	1581 (37.6%)				
Gestational Age (weeks)									
<37	35 (14.1%)	40 (15.0%)	0.77	658 (14.3%)	561 (13.3%)	0.19		0.91	0.45
≥ 37	214 (85.9%)	227 (85.0%)		3939 (85.7%)	3643 (86.7%)				
SGA <10th centile									
<37 weeks	12 (34.3%)	8 (20.0%)	0.16	60 (9.1%)	55 (9.8%)	0.68		<0.001	0.04
>37 weeks	46 (21.5%)	51 (22.5%)	0.81	432 (11.0%)	339 (9.3%)	0.02		<0.01	<0.01
Stillbirths	**N= 22 (4.1%)**			**N=83 (0.9%)**					
	11 (4.2%)	11 (4.0%)	0.87	41 (0.9%)	42 (1.0%)	0.61		<0.01	<0.01

Table 13: Adjusted Means of the Markers of Growth among PRETERM offspring of preeclamptic and normotensive women

Fetal measures	Male Normo [b] Mean (SE)	Male PE [b] Mean (SE)	Male Difference	Diff p	Female Normo [b] Mean (SE)	Female PE [b] Mean (SE)	Female Difference	Diff p	PExSEX Interaction p
Birth weight (g)	2674.34 (25.32)	2333.79(111.32)	-340.49	<0.01	2547.52 (25.67)	2487.40 (96.59)	-60.12	0.60	0.05
Crown-Heel Length (cm)[a]	48.02 (0.14)	46.35 (0.58)	-1.67	<0.01	47.36 (0.14)	47.11 (0.52)	-0.25	0.65	0.06
Head Circumference(cm)[a]	32.58 (0.08)	31.52 (0.34)	-1.06	<0.01	32.03 (0.09)	32.13 (0.31)	+0.10	0.77	0.02
Chest Circumference[a] (cm)	29.95 (0.13)	28.34 (0.53)	-1.61	<0.01	29.51 (0.12)	29.56 (0.44)	-0.05	0.91	0.01
Placental Weight (g) [a]	388.74 (3.96)	376.09 (18.68)	-12.65	0.51	378.29 (4.19)	381.38 (15.22)	+3.09	0.85	0.56
Ponderal Index[a]	2.422 (0.012)	2.335 (0.052)	-0.087	0.11	2.410 (0.013)	2.309 (0.047)	-0.101	0.04	0.78
Head- Chest Circumference Ratio[a]	1.093 (0.003)	1.119 (0.012)	+0.026	0.04	1.086 (0.003)	1.095 (0.010)	+0.009	0.36	0.28
Fetal Placental Weight Ratio[a]	6.94 (0.06)	6.90 (0.30)	-0.04	0.89	6.85 (0.07)	6.73 (0.24)	-0.11	0.65	0.91
	Male Normo [c] OR (95% CI)	**Male PE [c] OR (95% CI**		**p**	**Female Normo [c] OR (95% CI)**	**Female PE [c] OR (95% CI)**		**p**	
SGA (<10th percentile)	REFERENT	6.37 (2.90, 14.00)	-	<0.01	REFERENT	2.76 (1.18, 6.47)	-	0.02	0.08[d]
Synergy Index (95% CI)[e]	**0.38(0.00, 0.84)** Risk factors=female sex and preeclampsia								

Each fetal measure outcome variable was assessed in a separate regression model. Normo= Normotensive Offspring, PE = Preeclamptic Offspring
The reported p-values for interaction are for interaction in the additive scale unless otherwise specified.

[a] Reduced sample size due to missing measurements (missing=3% length, PI & head circumference, 10% chest circumference & HCC and 20% placental weight & FPR models).

[b] Derived from linear regression models adjusting for maternal race, socio-economic status (SES), pre-pregnancy body mass index (BMI), maternal smoking, and gestational age at delivery.

[c] Derived from logistic regression models adjusting for maternal race, socio-economic status (SES), pre-pregnancy body mass index (BMI), and smoking.

[d] p-value for interaction in the multiplicative scale

[e] A synergy index of 1.0 = no interaction, <1 indicates antagonism, and >1 indicates synergy.

Table 14: Adjusted Means of the Markers of Growth among TERM offspring of preeclamptic and normotensive women

Fetal measures	Male Normo [b] Mean (SE)	Male PE [b] Mean (SE)	Male Difference	Diff p	Female Normo [b] Mean (SE)	Female PE [b] Mean (SE)	Female Difference	Diff p	PExSEX Interaction p
Birth weight (g)	3220.85 (6.62)	3086.90 (28.49)	-133.95	<0.01	3130.46 (6.59)	2958.12 (26.59)	-172.34	<0.01	0.40
Crown Heel Length (cm) [a]	50.37 (0.04)	49.92 (0.15)	-0.45	<0.01	49.75 (0.04)	49.64 (0.15)	-0.11	0.47	0.12
Head Circumference (cm) [a]	34.04 (0.02)	33.78 (0.09)	-0.27	<0.01	33.50 (0.02)	33.20 (0.08)	-0.30	<0.01	0.76
Chest Circumference (cm) [a]	32.00 (0.03)	31.54 (0.12)	-0.46	<0.01	31.74 (0.03)	31.22 (0.12)	-0.52	<0.01	0.78
Placental Weight (g) [a]	430.55 (1.45)	418.87 (6.40)	-11.67	0.08	428.31 (1.51)	414.00 (6.20)	-14.31	0.03	0.78
Ponderal Index[a]	2.518 (0.004)	2.477 (0.019)	-0.041	0.04	2.542 (0.005)	2.434 (0.019)	-0.11	<0.01	0.02
Head- Chest Circumference Ratio[a]	1.066 (0.0008)	1.072 (0.004)	+0.006	0.09	1.057 (0.0008)	1.068 (0.003)	+0.011	<0.01	0.43
Fetal Placental Weight Ratio[a]	7.66 (0.02)	7.63 (0.09)	-0.03	0.76	7.49 (0.02)	7.38 (0.09)	-0.11	0.20	0.57
	Male Normo[c] OR (95% CI)	Male PE [c] OR (95% CI)		p	Female normo [c] OR (95% CI)	Female pe [c] OR (95% CI)		p	
SGA (<10th percentile)	REFERENT	2.22 (1.56, 3.16)	-	<0.01	REFERENT	2.92 (2.06, 4.12)	-	<0.01	0.30[d]
Synergy Index (95% CI)[e]	0.81 (0.32, 1.29) Risk factors=male sex and preeclampsia								

Each fetal measure outcome variable was assessed in a separate regression model.
The reported p-values for interaction are for interaction in the additive scale unless otherwise specified.
Normo= Normotensive Offspring, PE = Preeclamptic Offspring
[a] Reduced sample size due to missing measurements (missing=3% length, PI & head circumference, 12% chest circumference & HCC and 15% placental weight & FPR models).
[b]Derived from linear regression models adjusting for maternal race, socio-economic status (SES), maternal pre-pregnancy body mass index (BMI), maternal smoking, and gestational age at delivery.
[C]Derived from logistic regression models adjusting for maternal race, socio-economic status (SES), pre-pregnancy body mass index (BMI), and smoking.
[d] p-value for interaction in the multiplicative scale
[e] A synergy index of 1.0 = no interaction, <1 indicates antagonism, and >1 indicates synergy.

4.0 MANUSCRIPT 3: PREECLAMPSIA AND REDUCED PLACENTAL GROWTH

Manuscript in preparation

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4.1 ABSTRACT

The dimensions of the placenta may provide insight on the influence of placental under perfusion in the setting of preeclampsia. We studied the largest and smallest diameters, ratio of the diameters, placental thickness and placental weight among offspring of 735 preeclamptic and 21,185 normotensive, Black and White women enrolled in the Collaborative Perinatal Project (1959 -1965). Preeclampsia was associated with lower placental weight and diameters (test of trend, $p<0.01$ placental weight, $p<0.01$ smaller diameter and $p=0.04$ larger diameter, respectively). After additional adjustment for placental weight, only the smaller placental diameter retained an independent relationship with the risk of preeclampsia. When we investigated the relationship between placental weight and birthweight, we found that at lower (<430grams, $p<0.01$ interaction) and upper (>495 grams, $p=0.04$, interaction) ranges of placental weight, the rate of increase in birth weight with increasing placental weight was greater among offspring of preeclamptic vs. normotensive women. Similarly, we found that at lower ranges of both diameters (< 20cm, smaller diameter and < 23 cm, larger diameter), the rate of increase in birth weight with increasing diameters was greater among offspring of preeclamptic women (smaller diameter <12cm $p=0.02$; 12-16cm $p<0.01$; 16-20cm $p=0.01$; >20cm $p=0.88$; interaction respectively. Larger diameter <15cm $p<0.01$; 15-19cm $p<0.01$; 19-23cm $p<0.01$; >23cm $p=0.75$ interaction respectively). We also found that among the offspring of preeclamptic women, female offspring with smaller diameters above 20cm, had a reduction in birth weight while males did not ($p=0.02$, interaction). Similarly, we found that among offspring of normotensive women, female offspring with larger diameters above 23cm had a reduction in birth weight while males did not ($p<0.01$, interaction). Our study identified the smaller placental diameter as a potentially

important marker of placental growth or function beyond the information given by placental weight. This relationship may be different in the setting of preeclampsia and infant sex. These relationships may be beneficial in directing future research to interpret the relationship between placental structure/function, the pathophysiology of preeclampsia and the influence of fetal sex.

4.2 INTRODUCTION

Preeclampsia is a pregnancy specific disorder which is diagnosed by proteinuria and increased blood pressure that affects 3-5% of pregnancies (36). Unsuccessful remodeling of the spiral arteries in the uterus leads to reduced placental perfusion, the root cause of preeclampsia (32). Due to the risk of reduced nutrient and oxygen transfer during fetal development (30), reduced placental weight may result (25, 30, 164).

Placental weight is a gross measure of placental growth that summarizes the different dimensions of the placenta (28, 171). The placental weight, surface area, largest and smallest diameters and thickness are placental measures that can be routinely measured at birth. They were designed to capture different aspects of placental growth and the capacity of the placenta to exchange nutrients and oxygen with the fetus (27, 171, 177). The larger and smaller diameters determine the surface area available for spiral arteries in the uterine wall to perfuse the placenta (27). The disc thickness increases with increased branching of the villous capillary bed (27). The ratio of the diameters reflects the symmetry of placental growth (171), which is expected to be centripetal around the umbilical cord (171, 177). These dimensions of the placenta may provide more insight into the mechanisms of placental growth, beyond the information given vby placental weight. Additionally, sophisticated measures of placental growth can estimate the influence of placental under perfusion in the setting of preeclampsia.

A recent study that analyzed the 1934-44 Helsinki Birth cohort reported that preeclampsia was associated with a shorter small diameter that result in oval shaped placenta. The authors suggested that growth of the placenta may be polarized in the presence of

preeclampsia causing differential growth along the larger and smaller diameters (178). We investigated if this relationship was detectable in a more contemporary and racially diverse population. We also investigated which dimensions of placental growth (thickness, small and large diameters and ratio of the diameters) were reduced in association with preeclampsia.

We also hypothesized that in the presence of term preeclampsia infant birth weight would be more sensitive to changes in placental growth than that of the offspring of normotensive women, and that this may further vary according to fetal sex.

4.3 METHODS

4.3.1 Study Population

The subjects were pregnant women and their offspring who were enrolled in the Collaborative Perinatal Project (CPP), a prospective pregnancy cohort study of neurologic disorders and other conditions in children. The CPP collected data on pregnant women enrolled at 12 study centers during 1959 to 1965, about half of whom were African American (202, 203).

There were 54,681 singleton births in the Collaborative Perinatal Project. We limited the study to Black and White women. Hispanics and Asians were excluded because of small numbers (4.2%, n= 2,302). We excluded women with missing placental data (14%, n=7,422). The rates of missing data varied among Black (17.5%) vs. White women (9.3%), nonsmokers (14.6%) vs. smokers (10.7%) and among women with low (15.3%) vs. high socioeconomic status (8.5%). However this was driven by large numbers of women with missing placental data from specific sites (e.g. 70% of placental data was missing among women from the Tennessee site). We excluded women with certain abnormal shaped placentas (e.g. crescent shaped) and

multilobate placentas (n=1,779) because the measures of placental diameters and thickness would be inaccurate in these placentas (243). Gestational age at delivery was limited to 37 to 42 weeks and births with missing gestational ages were excluded (n=7,282). The analyses were limited to infants delivered at term because we had small numbers of preterm offspring of preeclamptic women with placental data (n=123). Gestational age was calculated based on the last menstrual period of the mother.

4.3.2 Definition of preeclampsia

Preeclampsia was defined as new-onset gestational hypertension and proteinuria, which resolved in the postpartum period. Gestational hypertension was defined as at least 2 measurements of systolic blood pressure >=140 mmHg and/or diastolic blood pressure >=90 mmHg with no prior occurrence before 24 weeks of gestation. Proteinuria was defined as 2 random urine dipsticks of 1+ protein or one dipstick of 2+ protein.

We excluded women with proteinuria only (n=1,696), elevated blood pressure before 24 weeks of pregnancy (n=3,399), chronic hypertension or transient hypertension of pregnancy (gestational hypertension without proteinuria) (206) (n=4,486). We also excluded women with missing hypertension status (n=183) and women diagnosed with diabetes (n=472). Pre-existing hypertension and diabetes may bias the effect of preeclampsia as the effect of these chronic diseases could influence the outcome.

4.3.3 Placental Growth Variables

The placentas were collected at delivery and measured by trained pathologists using a standardized protocol (244). The larger and smaller placental diameters (straight line segments that pass through the center of the placenta) were measured perpendicular to each other and recorded in centimeters. The thickness of the placental disc was measured by piercing the placenta with a needle calibrated in millimeters. After collecting measures of the placental dimensions, the placental membranes were then trimmed, the umbilical cord and any blood clots removed before weighing the placenta.

4.3.4 Statistical Analysis:

Chi-square tests were used to test for association between variables. Separate models for each placental dimension were used to evaluate the association between placental growth and preeclampsia. For the exposure, we created dummy variables of quartiles of the smaller diameter, larger diameter and the ratio of the diameters (smaller/larger). For placental thickness, we used the clinically significant cut points of 2.0-2.5cm as the range of normal placental thickness (177). The association of each quartile of placental dimension with preeclampsia was estimated using the largest measure as the referent for each placental measure and 2.0-2.5cm as the referent for placental thickness. The results were reported as estimated odds ratios and 95% confidence intervals. The likelihood ratio test was used to test for trends between decreasing placental dimensions and the risk of preeclampsia. A P value of <0.05 was considered statistically significant.

In normal pregnancy, the relationship between growth of the placental dimensions and birth weight is linear (179). We hypothesized that in the presence of preeclampsia there may be deviations from this linear relationship. Splines (piecewise polynomials that join smoothly at knots or breakpoints) can be used to assess departures from linearity. Linear regression models were fitted with linear splines to investigate the trends in the relationship between placental growth and birthweight among term offspring of normotensive and preeclamptic women. Three to four equally spaced knots were chosen to examine the linear trend between birth weight and placental growth. Statistical interactions between preeclampsia status and each piecewise spline were evaluated to determine if growth differed between offspring of normotensive and preeclamptic women. Similarly, interactions between infant sex and each piecewise spline were evaluated to determine if growth differed by fetal sex among offspring of normotensive and preeclamptic women. The linear spline curves in figures 1-2 were estimated by plotting the predicted mean birthweight vs. piecewise linear splines based on the unadjusted linear regression model for each placental dimension.

The covariates for multivariate analysis were chosen *a priori* from the literature. The variables selected were maternal smoking status at registration, socio-economic status (SES), age, parity and race, which were collected by self-report and maternal pre-pregnancy body mass index (BMI). Maternal smoking status at registration was coded as a yes/no dichotomous variable. The socioeconomic status variable was a combined score based upon education, occupation and income (202) and was categorized as low, middle and high socio-economic status. BMI was calculated from height (measured at enrollment) and self-reported pre-pregnancy weight. Maternal pre-pregnancy BMI was analyzed as a continuous variable. To address uncontrolled confounding due to study site, site was included as a confounder in the

analyses. Placental weight was moderately correlated with each placental diameter (r=0.50 and r=0.51 smaller and larger diameters respectively), and weakly correlated with placental thickness (r=0.34). There were no strong correlations among the placental measures. Therefore in order to investigate the relationships independent of placental weight, we performed secondary analyses adjusting the models for placental weight. Statistical analysis was performed with SAS 9.2 software (SAS Institute, Cary, N.C.). Linear splines were plotted using Sigma Plot 12.0 (Systat Software Inc, C.A.).

4.4 RESULTS

Preeclamptic women were more likely than normotensive women to be Black, non-smokers, of low socioeconomic status, primiparous, overweight and obese (Table 15). Offspring of normotensive vs. preeclamptic women had larger birthweights (P<0.01), heavier placentas (P=0.05) and consistent with this, greater mean larger (P<0.01) and smaller (P<0.01) diameters (Table 16). However, there were no differences in the mean thickness or ratio of the diameters. Among offspring of normotensive women, males had heavier placentas and as expected, greater mean larger (P<0.01) and smaller (P<0.01) placental diameters. However, there were no differences by sex in the mean thickness or ratio of the diameters. When we investigated the mean placental measures of offspring born to preeclamptic women, we found that only the mean larger diameter was greater in males vs. females (18.9 cm vs. 18.6 cm, P=0.04).

4.4.1 Risk of Preeclampsia by placental measure

Preeclampsia was more frequently associated with lower placental weights and lower placental diameters (Table 17; test of trend, $p<0.01$ placental weight, $p<0.01$ smaller diameter and $p=0.04$ larger diameter, respectively). We found no statistically significant relationship between the ratio of the diameters (ovalness of the placenta) and the risk of preeclampsia. However there was a pattern of increased risk of preeclampsia as the ratio of the diameters increased. After adjusting for confounders, we found no relationship between placental thickness and the odds of preeclampsia. After additional adjustment for placental weight, only the smaller placental diameter retained an independent relationship with the risk of preeclampsia.

4.4.2 Relationship between placental dimensions and birthweight among offspring of normotensive vs. preeclamptic women

Next we investigated the trends in the relationship between the placental dimensions and birth weight among offspring of normotensive and preeclamptic women. The rate of increase in birth weight with increasing placental weight was greater among offspring of preeclamptic vs. normotensive women with placental weights below 430grams ($p<0.01$ interaction) and above 495 grams ($p=0.04$, interaction) (**Figure 5**). Next we investigated if the relationship between the placental diameters as well as placental thickness were independent predictors of birthweight beyond that predicted by placental weight in offspring of preeclamptic vs. normotensive women. We found that the relationship between both diameters and birth weight were attenuated with the inclusion of placental weight (and placental thickness) but retained an independent relation with birth weight (**Figure 6** A and B). We found that at lower ranges of both diameters (< 20cm,

smaller diameter and < 23 cm, larger diameter), the rate of increase in birth weight with increasing diameters was greater among offspring of preeclamptic women (smaller diameter <12cm p=0.02; 12-16cm p<0.01; 16-20cm p=0.01; >20cm p=0.88; interaction respectively; larger diameter <15cm p<0.01; 15-19cm p<0.01; 19-23cm p<0.01; >23cm p=0.75 interaction respectively). In contrast to the diameters however, the relationship between placental thickness and birthweight as well as ratio of the diameters and birth weight did not differ between offspring of normotensive and preeclamptic women.

4.4.3 Relationship between the smaller and larger placental diameters and birth weight by infant sex

We then investigated if the trends in the relationship between both placental diameters and birth weight differed by sex among offspring of normotensive and preeclamptic women. After adjustment for placental weight, we found that among the offspring of preeclamptic women, female offspring with smaller diameters above 20cm, had a reduction in birth weight while males did not (p=0.02, interaction) (Figure 7A). Similarly, we found that among offspring of normotensive women, female offspring with larger diameters above 23cm had a reduction in birth weight while males did not (p<0.01, interaction) (Figure 7B). None of the other placental measures were different between male and female fetuses with or without preeclampsia.

4.5 DISCUSSION

Our study identified that the smaller placental diameter is a potentially important marker of placental growth or function beyond the information given by placental weight. We confirmed a previous observation of lower placental weight in preeclampsia. After controlling for the influence of placental weight, we found that the smaller but not the larger placental diameter was an independent predictor of preeclampsia. In addition, the smaller diameter also had an independent relationship with birth weight beyond that predicted by placental weight. This relationship may be different in the setting of preeclampsia and infant sex.

The relationship between the smaller diameter of the placenta and preeclampsia remained statistically significant after adjustment for the ratio of the diameters, suggesting that this relationship was independent of placental shape. Unsuccessful remodeling of the spiral arteries in the uterus leads reduced placental perfusion, the root cause of preeclampsia (32). The larger and smaller diameters indicate the area available for spiral arteries in the uterine wall to perfuse the placenta (27). We are unable to explain the association between preeclampsia and the smaller but not the larger placental diameter. We agree with Kajantie (178) in speculating that the biologic processes responsible for placental perfusion may influence growth or function in the planes of the placental diameters differently.

Our findings of the association between preeclampsia and reduced placental diameters are consistent with a study by Kajantie who investigated these associations among infants born in 1934-1944 as part of the Helsinki Birth Cohort (178). Unlike Kajantie, we did not find an excess of ovoid placentas or thicker placentas in preeclamptic pregnancies. The Helsinki birth cohort is

25 years older than the Collaborative Perinatal cohort and the infants were born during the Second World War during a time of severe food shortages (163). Poor maternal nutrition may have intensified the influence of reduced placental growth in the setting of preeclampsia and thus may explain our discrepant results. Our study utilized a racially diverse population, with potentially better perinatal nutrition than the Helsinki Birth cohort and thus may be more relevant to a modern population.

We found that the relationship between placental growth and birth weight differed between normotensive and preeclamptic pregnancies. We found higher rates of increase in birth weight at lower and higher placental weight for offspring of preeclamptic vs. normotensive women. This may indicate that at the extremes of placental weight, the placenta in preeclampsia may be more efficient at translating placental weight to fetal weight. Among the placental diameters (independent of placental weight), we found a relationship similar to that observed between the lower placental weight and birth weight. For lower diameters in both planes of the placental disc in preeclamptic pregnancies, the infant birthweight increased more rapidly compared to similar placental diameters in normotensive pregnancies. This observed differential growth at the lower range placental weights and placental diameters suggests a "more efficient" small placenta in preeclampsia. This observation is consistent with a prior observation of normal amino acid transport in SGA infants from preeclamptic pregnancies which is reduced with SGA without preeclampsia (245).

Our findings suggest that the relationship between growth of the smaller placental diameter and birth weight may be different by infant sex. In preeclamptic pregnancies, there appeared to be a threshold beyond which birthweight was reduced for female but not for male offspring as the smaller diameter increased. A similar relationship was observed with the larger

placental diameter of offspring of normotensive women. This is consistent with large cohort studies that suggest the placentas of male infants are more efficient at translating placental growth to fetal growth than placentas of females (103, 179, 246). Eriksson and Misra both reported higher mean fetal placental ratio (birthweight(g)/placental weight(g)) for males vs. females ($p<0.01$) (103, 179), and a greater rate of increase in birth weight for female vs. male infants at higher ranges of placental area (179). Our findings also suggest that growth of the placental diameters may be influenced through different biological pathways in the setting of preeclampsia and by infant sex.

Our study had several strengths. The Collaborative Perinatal Project (CPP) provided a large dataset, which allowed the exploration of the relatively rare adverse pregnancy outcome-preeclampsia. The CPP also included large numbers of placental measures collected according to standardized research protocols. These methods of placental measurements have not changed considerably and thus are still relevant to current measurements. Placentas for research are typically collected for cases of adverse outcome. However, the CPP is one of only a few large studies of this scale with measurements of placentas from normal and adverse pregnancy outcomes in the United States. The management of preeclampsia has changed since the 1960s. In the 1960's there were fewer interventions for fetal indications. This allowed us to better investigate the natural progression of the disease on placental growth than would be possible with a more modern cohort.

The study was limited to placental measures that were taken at birth and therefore do not reflect growth rate throughout pregnancy. Placental thickness was measured at the center of the placenta. As a result, this measure may not indicate the thickness throughout the placenta, especially in placentas with irregular or non-uniformed growth. The placental diameters may not

capture true growth of irregular shaped placentas with non-uniformed growth. We attempted to limit this bias by removing multilobate and irregular shaped placentas from our analyses.

Our study provides evidence to suggest that the smaller diameter of the placenta is an independent predictor of preeclampsia beyond the influence predicted by placental weight. We also found a disparity in the rate of increase in birthweight at the lower range of placental weight and placental diameters. We believe that this relationship may be indicative of catch-up (fetal) growth for infants with small placentas in preeclamptic pregnancies. Our results also imply that the relationship between the growth of the smaller diameter and birth weight may operate through different biological pathways by sex and is different in the setting of preeclampsia. Moreover, it suggests that the smaller diameter may be a potential biologically relevant marker of placental growth/function that may provide information beyond that given by placental weight. These findings indicate the importance of incorporating the placental diameters and infant sex in the study of mechanisms relating placental and fetal growth. These relationships may be beneficial in directing future research to interpret the relationship between placental structure/function, the pathophysiology of preeclampsia and the influence of fetal sex.

4.6 Tables and Figures

Table 15: Baseline Characteristics of the population of women (n=21,920) (Primiparous and Multiparous)

Characteristics	Women (n=21,920)		
	Normotensive (N=21185) n(%)	Preeclamptic (N=735) n(%)	P-value
Maternal Age			
<20	4774 (22.5%)	253 (34.4%)	<0.01
20-29	12937 (61.1%)	345 (46.9%)	
30-39	3474 (16.4%)	137 (18.6%)	
Maternal Race			
White	12004 (56.7%)	323 (44.0%)	<0.01
Black	9181 (43.3%)	412 (56.0%)	
Infant Sex			
Females	10403 (49.1%)	365 (49.7%)	0.77
Males	10782 (50.9)	370 (50.3%)	
Smoking			
Nonsmoker	10660 (50.3%)	439 (59.7%)	<0.01
Smoker	10525 (49.7%)	296 (40.3%)	
BMI (kg/m^2)			
Underweight (<18.5)	2108 (10.0%)	40 (5.4%)	<0.01
Normal (18.5-24.9)	15165 (71.6%)	461 (62.7%)	
Overweight (25.0- 29.9)	2985 (14.0%)	140 (19.1%)	
Obese (>30)	927 (4.4%)	94 (12.8%)	
Maternal SES score			
1 (low)	7369 (34.8%)	346 (47.1%)	<0.01
2 (medium)	6464 (30.5%)	223 (30.3%)	
3 (high)	7352 (34.7%)	166 (22.6%)	
Parity			
Primiparous	6371 (30.1%)	351 (47.8%)	<0.01
Multiparous	14788 (69.9%)	383 (52.2%)	
Birthweight	**Mean (SE)**	**Mean (SE)**	
	3233.99 (3.22)	3144.70 (17.29)	<0.01

Table 16: Mean values of placental measurements in preeclamptic and normotensive pregnancies (Term births)

Placental Dimension	Preeclampsia N=735						Normotensive N=21185					
	All Mean (SE)	Males		Females		P-Value	All Mean (SE)	Males		Females		P-value
		n	Mean (SE)	n	Mean (SE)			n	Mean (SE)	n	Mean (SE)	
placental weight	432.08 (3.42)*	357	434.27 (4.83)	356	429.89 (4.84)	0.52	438.95 (0.64)	10454	441.86 (0.89)	10068	435.94 (0.91)	<0.01
Larger diameter	18.73 (0.08)*	370	18.88 (0.11)	365	18.57 (0.11)	0.04	18.94 (0.14)	10782	19.02 (0.02)	10403	18.87 (0.02)	<0.01
Smaller diameter	16.26 (0.07)*	369	16.31 (0.10)	365	16.21 (0.10)	0.45	16.47 (0.13)	10782	16.54 (0.02)	10403	16.40 (0.02)	<0.01
Ratio of Diameters (%)[a]	86.92 (0.32)	370	86.50 (0.44)	365	87.34 (0.45)	0.18	87.25 (0.06)	10782	87.26 (0.08)	10403	87.24 (0.08)	0.84
Thickness	2.18 (0.02)	370	2.19 (0.02)	365	2.18 (0.03)	0.74	2.20 (0.003)	10782	2.20 (0.005)	10403	2.20 (0.005)	0.39

a=Ratio of the small/large diameter (%)
*= $p \leq 0.05$ of comparison of all Preeclamptic vs. all Normotensive Mean Placental Dimension

Table 17: Crude and Adjusted models showing risk of Preeclampsia by placental measure

Marker	Term Normo (n)	Term PE (n)	Crude OR (95% CI)	*Adjusted OR (95% CI)	** Additionally adjusted for placental weight OR (95% CI)
Placental weight (g)					
<375	5169	221	1.33 (1.09, 1.62)	1.39 (1.12, 1.72)	
375-430	4885	149	0.96 (0.77, 1.19)	1.01 (0.80, 1.27)	
430-495	5434	181	1.04 (0.85, 1.28)	1.07 (0.86, 1.34)	
>495	5164	166	Ref	Ref	
Test of trend				P<0.01	
Small diameter (cm)					
<15	6286	255	1.28 (1.06, 1.55)	1.35 (1.11, 1.65)	1.27 (1.01, 1.59)
16	4561	161	1.11 (0.90, 1.38)	1.16 (0.93, 1.44)	1.12 (0.89, 1.41)
17,18	4406	131	0.94 (0.75, 1.18)	0.98 (0.78, 1.23)	0.94 (0.74, 1.20)
>18	5932	187	Ref	Ref	Ref
Test of trend				P<0.01	P=0.06
Large Diameter (cm)					
<18	4157	131	1.25 (1.00, 1.56)	1.28 (1.02, 1.61)	1.18 (0.90, 1.54)
18,19	3645	124	1.20 (0.84, 1.39)	1.18 (0.86, 1.32)	1.14 (0.82, 1.30)
20	8426	285	1.01 (0.87, 1.32)	1.01 (0.83, 1.37)	0.98 (0.78, 1.32)
>20	4957	195	Ref	Ref	Ref
Test of trend				P=0.04	P=0.24
Ratio of diameters Small/Large (%)					
<81.82	4887	178	1.14 (0.92, 1.42)	1.17 (0.94, 1.46)	1.12 (0.90, 1.40)
81.82-88.24	4771	171	1.12 (0.90, 1.40)	1.14 (0.91, 1.42)	1.08 (0.87, 1.36)
88.24–94.12	6393	222	1.09 (0.89, 1.34)	1.10 (0.89, 1.35)	1.07 (0.86, 1.32)
>94.12	5134	164	Ref	Ref	Ref
Test of trend				P=0.54	P=0.78
Thickness (cm)					
>2.5	2962	104	1.09 (0.88, 1.35)	1.10 (0.89, 1.37)	1.17 (0.93, 1.48)
2.0-2.5	14238	459	Ref	Ref	Ref
<2.0	3985	172	1.34 (1.12, 1.60)	1.16 (0.96, 1.40)	1.11 (0.92, 1.34)
Test of trend				P=0.26	P=0.28
*Models adjusted for maternal race, pre-pregnancy BMI, age, smoking status at registration, infant sex, parity, gestational age at delivery and site of study.					

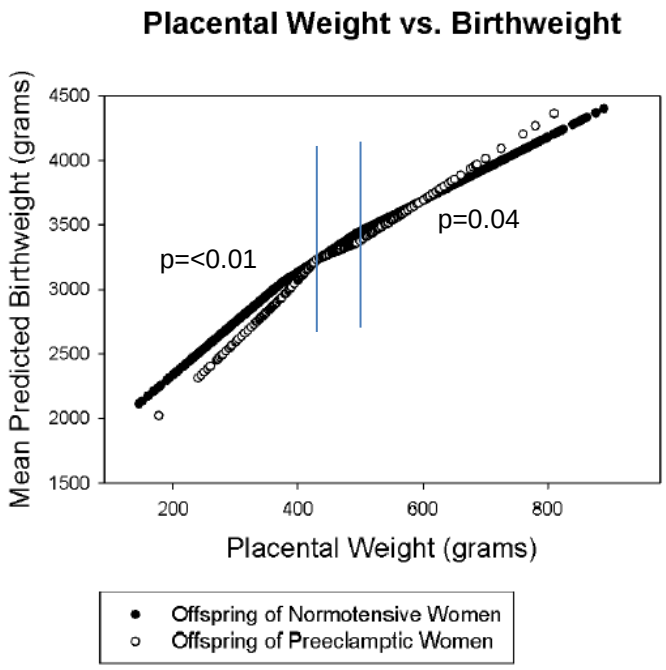

Figure 5: Mean birth weight vs. Placental weight among offspring of Preeclamptic vs. Normotensive women. Curves were estimated by calculating predicted mean birthweight based on unadjusted spline regression models

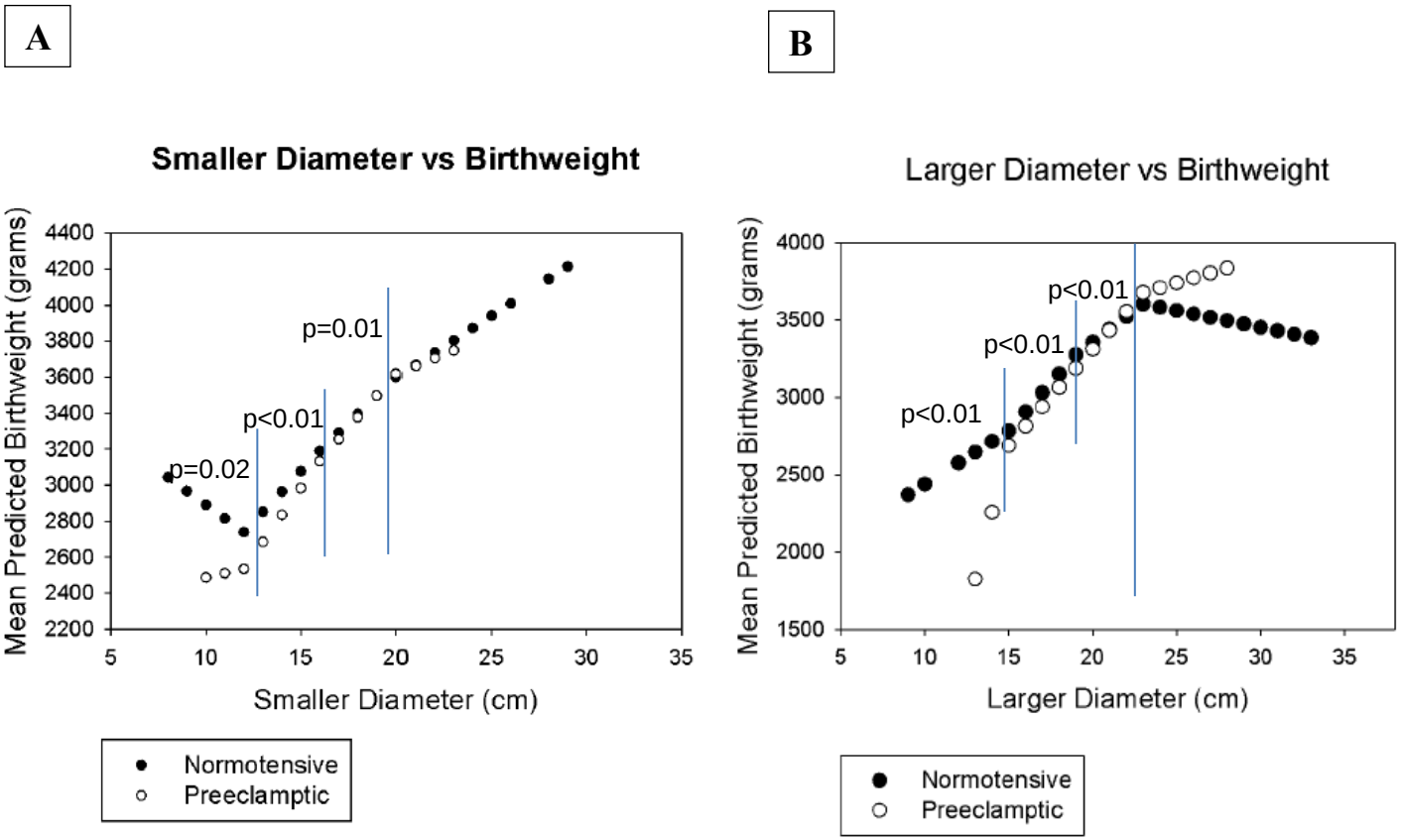

Figure 6: Mean birth weight vs. Smaller and Larger diameters among offspring of Preeclamptic vs. Normotensive women. Curves were estimated by calculating predicted mean birthweight based on unadjusted spline regression models

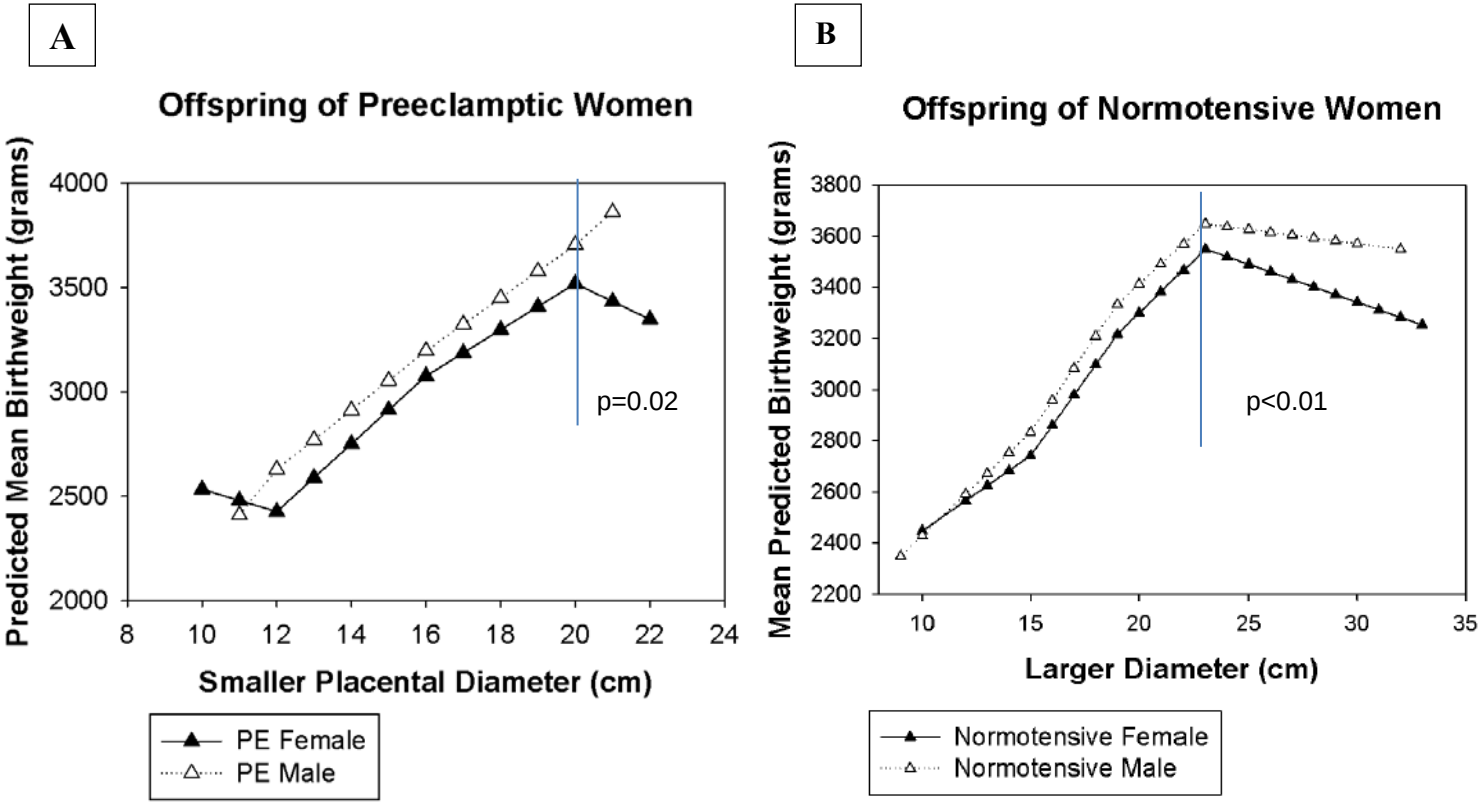

Figure 7: Mean birth weight vs. Smaller placental diameter by infant sex among offspring of Preeclamptic (A) women. Mean birth weight vs. Larger placental diameter by infant sex among offspring of Normotensive (B) women. Curves were estimated by calculating predicted mean birthweight based on unadjusted spline regression models

5.0 CONCLUSION

In this study we used data from the National Collaborative Perinatal Project and investigated 3 important features: 1) the influence of fetal sex and maternal race on maternal pre-pregnancy body mass index, smoking and socio-economic status; 2) the influence of preeclampsia on fetal growth and the influence of infant sex; 3) the influence of preeclampsia on placental growth and the interaction with infant sex. These areas have not been extensively studied. Our results add to the growing literature on the differential influence of fetal sex and race in pregnancy outcome.

Despite the anthropometric measures indicating reduced growth among Black vs. White infants born to normotensive women, the differential rate of SGA between Black and White infants was not evident using race adjusted growth charts. Given the well-established lower mean birthweight and higher mortality rate among Black vs. White infants, it is reasonable to expect that growth restriction and not physiology contributes to these higher rates in Blacks. Our findings, which are consistent with the large body of evidence that offspring of Black women are at greater risk for fetal growth restriction than offspring of White women (142, 233, 234), supports the use of non-race adjusted growth curves.

Our findings lead us to agree with Eriksson and Clifton who theorize that female and male fetuses have different responses to the same maternal environment (94, 103). In our first paper we found that in the absence of in utero insults, females were more responsive to the maternal environment. They had greater increases in fetal growth in response to increasing maternal pre-pregnancy BMI. In our second and third papers we found that females were also

more responsive in the setting of mild insults. In the setting of term preeclampsia, female infants had lower ponderal index. In addition, the female infants also had reduced birth weight at higher ranges of the smaller diameter while boys had continued growth. This finding is consistent with an asthma study by Murphy which found that among pregnant women with mild asthma who did not use inhaled steroids to treat asthma during pregnancy, female infants had greater reductions in growth, while males were unaffected (101).

The findings in our second paper further agree with the theories of Clifton and Eriksson (94, 103) which suggest that the inadequate response of male infants to minor insults makes them more susceptible to severe insults. In the setting of preterm preeclampsia, females again had better adaptation to the maternal environment. Because males are less responsive and adapt less readily to the maternal environment, the severe insults of preterm preeclampsia had a greater negative impact on male vs. female infants. This is again consistent with another asthma study by Murphy which found that among pregnant asthmatic women with severe exacerbations that required hospitalization, male infants had greater reductions in birth weight (102).

Despite the fact that the growth reductions in our study were subtle, our results raise the possibility that fetal and placental growth may be influenced through different biological pathways by fetal sex. The one-time measures of fetal and placental growth at birth in our study may have limited our findings. However our findings still support the need for further research into the influence of sex and race on fetal and placental growth and birth outcomes, especially in the setting of preeclampsia. They further suggest that mechanistic studies of fetal growth should investigate the influence in each sex. Future studies are needed to clarify the influence of infant sex and race on both growth promotion and growth reduction during fetal development. This may be beneficial in creating future biological research to interpret the pathways involved in the

pathophysiology of preeclampsia, as well as help to explain the mechanisms for sex differences in pregnancy outcomes and fetal origins of adult disease.

5.1 FUTURE RESEARCH

Our findings of differential fetal growth by fetal sex and race in normotensive pregnancies and differential fetal and placental growth in preeclamptic pregnancies warrant further investigation. Studies of differential outcome of fetal sex and race on birth outcomes have been limited to one time measures at birth. Future studies to clarify the influence of preeclampsia, sex and race on growth promotion/reduction during fetal development will need to identify the relevant biological pathways by 1) designing studies with large and diverse sample sizes, as many studies of preeclampsia are small and/or lack ethnic diversity; 2) recruiting women early in pregnancy in order to follow them and obtain measures throughout pregnancy; 3) recognizing the value of sex differences and analyze males and females separately; 4) exploring sex specific effects of biological effectors such as placental growth factors as well as other endothelial growth factors to identify potential biological pathways; 5) obtaining ultrasound measures of fetal and placental anthropometry throughout pregnancy; 6) exploring the long term effects on pediatric growth.

Identification of these pathways may be beneficial in creating future biological research to increase growth among infants that are identified to be at risk for growth restriction or who are found to have growth restriction early in utero. Those findings may bring us closer to understanding the pathophysiology of preeclampsia as well as explain why morbidity and mortality rates differ by sex in pregnancy complications. These pathways may also help to explain the mechanisms for sex differences in birth outcomes as well as fetal origins of adult

disease. Continuous ultrasound measures of growth in utero may also prove to be beneficial in creating new scales of growth to identify and distinguish infants who are constitutionally small from those who are growth restricted.

5.2 APPLICATION TO PUBLIC HEALTH

Neonatal and perinatal mortality and morbidity resulting from fetal growth restriction and preeclampsia, result in great economic and social burdens to the national health care system and the families involved. Our study contributes new insight into the complex relationship among preeclampsia, fetal growth, placental growth, infant sex and maternal race. Not all small-for-gestational age (SGA) infants are small due to impaired growth (e.g. small parents have appropriately grown small infants) and not all growth restricted infants are identified using SGA criteria. Infants may fall within the normal range of birth weight but show metabolic, hematologic, and neurologic characteristics as seen in growth-restricted infants (235). Our study adds new contribution by examining in addition to SGA, several other indicators of fetal growth: ponderal index, head to chest circumference ratio and fetal placental ratio to capture reduced fetal growth. Additionally, these measures provide support that using non-race adjusted weight for gestational age tables are more appropriate than race adjusted tables.

Our study also contributes important information on racial disparities in smoking in pregnancy and fetal growth. We also provided evidence to suggest that the smaller diameter may be a potential biologically relevant marker of placental growth/function that may provide information beyond that given by placental weight. In a more general sense this provides evidence that measurement of placental shape can yield information not provided by placental

weight alone. Our work builds on the emerging evidence that in utero insults negatively impact fetal and placental growth and additionally may also be influenced by infant sex.

Identification of these biological pathways that operate differently by maternal factors, smoking, race, preeclampsia and infant sex may be beneficial in creating future biological research to increase growth among infants that are identified to be at risk for growth restriction. Based on the Fetal Origins of Adult Disease Hypothesis, this may also prove to be invaluable in reducing the rates of later life diseases such as hypertension, cardiovascular disease, chronic kidney disease and diabetes in future generations.

APPENDIX A: SUPPLEMENTARY TABLES FOR MANUSCRIPT 1

Table 18: Influence of 1 kg/m2 increase in BMI by Fetal sex and Race on each Growth Measure among preterm infants.

Maternal Pre-pregnancy BMI	Male [b] Beta coefficient ±SE	Female [b] Beta coefficient ±SE	SEXxBMI Interaction p	Black [c] Beta coefficient ±SE	White [c] Beta coefficient ±SE	RACExBMI Interaction p
Birthweight (g)	29.92 (8.50)*	19.92 (8.63)†	**0.39**	20.36 (7.17)*	33.66 (11.34)*	0.38
Crown Heel Length (cm)	0.167 (0.047)*	0.127 (0.048)*	**0.43**	0.135 (0.041)*	0.151 (0.060)†	**0.89**
Head Circumference (cm)	0.109 (0.028)*	0.081 (0.029)*	**0.43**	0.091 (0.025)*	0.097 (0.034)*	0.94
Chest Circumference (cm)	0.104 (0.047)†	0.083 (0.039)†	**0.73**	0.092 (0.035)*	0.098 (0.062)	0.98
Placental Weight (g)	4.384 (1.310)*	3.627 (1.335)*	**0.79**	4.143 (1.171)*	3.810 (1.557)†	0.80
Ponderal Index	0.007 (0.004)	0.004 (0.004)	**0.65**	0.005 (0.004)	0.009 (0.005)	**0.52**
Head- Chest Circumference Ratio	-0.0003 (0.001)	-0.0003 (0.0009)	**0.77**	0.0002 (0.0008)	-0.0007 (0.0014)	0.64
Fetal Placental Weight Ratio	-0.004 (0.021)	-0.01 (0.02)	0.40	-0.023 (0.019)	-0.020 (0.025)	0.21
	Male [d] OR (95% CI)	**Female [d] OR (95% CI**	**P Interaction**	**Black [e] OR (95% CI)**	**White [e] OR (95% CI)**	**P Interaction**
SGA (<10th percentile)	0.95 (0.87, 1.06)	0.97 (0.88, 1.08)	**0.89**	0.97 (0.89, 1.06)	0.94 (0.83, 1.06)	0.64

*=p<0.01, †=p≤0.05

[b]Derived from linear regression models adjusting for maternal age, race, maternal smoking, SES and infant gestational age at delivery.

[c]Derived from linear regression models adjusting for infant sex and gestational age at delivery and maternal age, SES and smoking.

[d]Derived from logistic regression models adjusting for maternal age, race, socioeconomic status (SES), and smoking.

[e]Derived from logistic regression models adjusting for infant sex infant and maternal age, smoking and socioeconomic status (SES).

Table 19: Differential Influence of Smoking by race among preterm infants.

	Non Smoker Mean (SE)	Smoker Mean (SE)		Nonsmoker Mean (SE)	Smoker Mean (SE)		SMOKER x RACE Interaction p
	Black[b]		difference	White[b]		difference	
Birthweight (g)	2648.77 (28.56)	2528.15 (34.52)	-120.63*	2690.65 (54.44)	2596.68 (43.29)	-93.97	0.74
Crown Heel Length (cm)	47.95 (0.16)	47.23 (0.19)	-0.72*	47.99 (0.30)	47.59 (0.24)	-0.40	0.47
Head Circumference (cm)	32.47 (0.09)	32.00 (0.11)	-0.47*	32.50 (0.18)	32.29 (0.15)	-0.21	0.33
Chest Circumference (cm)	29.96 (0.13)	29.45 (0.16)	-0.51*	29.68 (0.27)	29.63 (0.22)	-0.05	0.25
Placental Weight (g)	375.19 (4.70)	388.33 (5.32)	+13.14	385.73 (8.01)	392.91 (6.67)	+7.18	0.63
Ponderal Index	2.408 (0.014)	2.400 (0.017)	-0.008	2.427 (0.026)	2.453 (0.021)	+0.026	0.38
Head- Chest Circumference Ratio	1.088 (0.003)	1.089 (0.004)	+0.001	1.097 (0.006)	1.094 (0.005)	-0.004	0.58
Fetal Placental Weight Ratio	7.159(0.076)	6.701 (0.086)	-0.458*	6.919 (0.131)	6.616 (0.108)	-0.303	0.44
	Black[c] OR (95% CI)			White[c] OR (95% CI)			
SGA (<10th percentile)	REFERENT	1.92 (1.17, 3.15)		REFERENT	1.51 (0.75, 3.02)		0.69

*= p<0.01, †= p≤0.05

[b]Derived from linear regression models adjusting for infant sex, gestational age at delivery and maternal age, pre-pregnancy body mass index (BMI) and SES.

[c]Derived from linear regression models adjusting for infant sex, maternal age, pre-pregnancy body mass index (BMI), and socioeconomic status.

APPENDIX B: SUPPLEMENTARY TABLES FOR MANUSCRIPT 3

Table 20: Results of unadjusted linear regression analysis with linear splines of placental predictors vs. birthweight in offspring of preeclamptic vs. normotensive women.

Variables	Slope Normo[a]	Slope PE [b]	Interaction (p)
Placental weight (g)			
<375	4.08	4.63	<0.01
375	3.09	5.30	<0.01
430	3.26	1.99	0.05
495	2.44	3.19	0.02
Large diameter (cm)			
<15	68.94	431.73	<0.01
15	122.77	124.96	<0.01
19	82.32	122.00	<0.01
23	-21.75	31.86	0.03
Small diameter (cm)			
<12	-76.30	24.42	0.13
12	112.86	149.57	<0.01
16	102.59	121.31	0.02
20	68.18	43.09	0.72
Smaller/larger diameter ratio (%)			
<60	3.47	1.03	0.32
60	0.27	21.22	0.02
75	3.33	9.36	0.12
85	-0.13	-3.46	0.88
95	-2.99	-17.48	0.26
Thickness (cm)			
<1.5	78.58	202.92	0.60
1.5	259.36	275.17	0.99
2.5	161.83	23.51	0.45
3.5	-102.38	156.16	0.07

[a]normo= offspring of normotensive women, [b]PE= offspring of preeclamptic women

Table 21: Results of adjusted linear regression analysis with linear splines of placental predictors vs. birthweight in offspring of preeclamptic vs. normotensive women**.**

	Adjusted			**Additionally adjusted for placental weight**			**Additionally adjusted for placental weight and thickness**
Variables	Slope Normo[a]	Slope PE[b]	Interaction (p)	Slope Normo[a]	Slope PE[b]	Interaction (p)	Interaction (p)
Placental weight (g)							
<375	3.92	4.53	<0.01				
375	2.79	4.83	<0.01				
430	3.16	1.91	0.05				
495	2.28	2.86	0.04				
Large diameter (cm)							
<15	67.84	430.94	<0.01	47.85	374.09	<0.01	<0.01
15	113.04	115.77	<0.01	46.16	51.19	<0.01	<0.01
19	77.55	121.87	<0.01	20.28	65.36	<0.01	<0.01
23	-17.41	-14.07	0.14	-11.08	-77.66	0.73	0.75
Small diameter (cm)							
<12	-69.12	-15.19	0.22	-30.77	140.72	0.02	0.02
12	103.94	136.61	<0.01	48.76	80.39	<0.01	<0.01
16	99.05	114.55	0.04	30.68	39.06	0.01	0.01
20	68.62	14.06	0.91	22.77	-54.55	0.89	0.88
Smaller/larger diameter ratio (%)							
<60	4.45	13.49	0.28	2.77	9.63	0.30	
60	-0.48	16.24	0.03	0.85	15.49	0.06	
75	4.09	9.04	0.24	2.01	2.41	0.80	
85	0.85	-4.60	0.64	1.06	-5.80	0.26	
95	-2.93	-6.78	0.46	-2.66	5.35	0.87	
Thickness (cm)							
<1.5	92.83	354.83	0.34	65.15	218.53	0.45	
1.5	174.63	180.41	0.96	-35.41	-73.32	0.93	
2.5	162.68	12.12	0.29	-88.49	-168.62	0.27	
3.5	-100.35	75.39	0.25	27.00	-540.67	0.10	

Adjusted for infant sex, maternal race, pre-pregnancy BMI, age, Socio-economic status, smoking, parity, site, (placental weight), (thickness). [a]normo= offspring of normotensive women, [b]PE= offspring of preeclamptic women

Table 22 : Results of unadjusted linear regression analysis with linear splines of placental predictors vs. birthweight in male vs. female offspring of preeclamptic women.

Variables	Slope Males (PE)	Slope Females (PE)	Interaction (p)
Large diameter			
<15	406.78	461.42	0.70
15	113.75	132.95	0.67
19	134.21	99.51	0.99
23	-51.46	182.88	0.10
Small diameter			
<12	219.01	-53.58	0.63
12	141.76	162.27	0.90
16	126.92	110.78	0.35
20	156.26	-85.73	0.08

Table 23: Results of adjusted linear regression analysis with linear splines of placental predictors vs. birthweight in male vs. female offspring of preeclamptic women.

Variables	Slope Males	Slope Females	Interaction (p)	additionally adjusted for placental weight interaction (p)
Large diameter				
<15	345.58	511.23	0.36	0.63
15	104.33	128.84	0.71	0.88
19	148.38	87.87	0.41	0.51
23	-89.31	123.27	0.25	0.92
Small diameter				
<12	299.47	-48.40	0.49	0.81
12	126.63	144.53	0.93	0.67
16	129.88	100.71	0.13	0.10
20	128.61	-120.42	0.03	0.02
Adjusted for maternal race, pre-pregnancy BMI, age, socio-economic status, smoking, parity, site, (placental weight).				

Table 24: Results of unadjusted linear regression analysis with linear splines of placental predictors vs. birthweight in male vs. female offspring of normotensive women.

Variables	Slope Males	Slope Females	Interaction (p)
Large diameter (cm)			
<15	80.77	58.82	0.43
15	126.38	118.52	0.33
19	78.12	83.39	0.93
23	-10.83	-29.67	0.26
Small diameter (cm)			
<12	-81.27	-52.88	0.79
12	117.20	106.47	0.41
16	97.30	105.35	0.10
20	51.21	93.30	0.05

Table 25: Results of adjusted linear regression analysis with linear splines of placental predictors birthweight in male vs. female offspring of normotensive women.

Variables	Slope Males	Slope Females	Interaction (p)	Additionally adjusted for placental weight interaction (p)
Large diameter (cm)				
<15	77.14	58.66	0.49	0.85
15	117.22	109.65	0.48	0.15
19	74.06	80.79	0.85	0.51
23	-5.85	-28.17	0.16	<0.01
Small diameter (cm)				
<12	-77.41	-58.60	0.83	0.86
12	108.42	98.90	0.57	0.72
16	94.91	103.50	0.11	0.20
20	54.80	93.17	0.06	0.44
Adjusted for maternal race, pre-pregnancy BMI, age, socio-economic status, smoking, parity, site, (placental weight).				

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