LOW BIRTHWEIGHT DELIVERY AND LONG TERM MATERNAL CARDIOVASCULAR HEALTH

by

JIA XU

BMed, Peking University, China, 2004

MS, Michigan State University, 2007

MS, University of Pittsburgh, 2012

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This dissertation was presented

by

JIA XU

It was defended on

July 29, 2015

and approved by

Dissertation Advisor:
Janet M. Catov, PhD
Associate Professor
Department of Obstetrics, Gynecology & Reproductive Sciences
Magee Womens Hospital
Department of Epidemiology
Graduate School of Public Health
University of Pittsburgh

Emma Barinas-Mitchell, PhD
Assistant Professor
Department of Epidemiology
Graduate School of Public Health
University of Pittsburgh

Ada O. Youk, PhD
Associate Professor
Department of Biostatistics
Graduate School of Public Health
University of Pittsburgh

Lewis H. Kuller, MD, DrPH
Distinguished University Professor of Public Health
Department of Epidemiology
Graduate School of Public Health
University of Pittsburgh

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Janet M. Catov, PhD

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Jia Xu, PhD

University of Pittsburgh, 2015

Objective: The objective of this dissertation is to provide insights into potential underlying pathways linking a prior low-birth-weight (LBW) delivery to long term maternal cardiovascular health.

Methods: This dissertation comprises three individual studies. The first study evaluates blood pressure and hypertension in women who reported a prior preterm (PTB) or small-for-gestational-age (SGA) LBW delivery in the National Health and Nutrition Examination Survey 1999-2006 (n=6,307). The second study examines associations between PTB and maternal interleukin-6 (IL-6) and C-reactive protein (CRP) concentrations (n=361) eight years after delivery in the Women and Infant Study of Healthy Hearts study (WISH study). The third study is an exploratory study within the WISH study to examine the relations between a prior PTB and adiposity measures eight years after delivery. It also investigates how adiposity measures may contribute to the associations between a prior PTB and subsequent cardiometabolic and inflammatory risk factors.

Results: The major findings of the study are 1) there was a positive association between a preterm-LBW and hypertension (adjusted OR=1.39, 95% confidence interval (CI) 1.02-1.90). Non-Hispanic African American women had increased risk of hypertension following SGA-LBW delivery (adjusted OR=2.09, 95% CI 1.12-3.90); 2) women with a previous spontaneous

PTB had higher IL-6 concentrations compared to women with term delivery (2.18 pg/ml vs. 1.82 pg/ml, $p<0.05$), after adjusting for potential confounding variables; 3) visceral adipose tissue (VAT) was higher in women with a prior PTB vs. term delivery among non-obese women after adjustment for body mass index (14.1 ± 7.5 cm^2, $p=0.07$). VAT may be a potential mediator of the association between PTB and elevated triglycerides later in life (95% confidence interval for the indirect effect of PTB on triglyceride through VAT: 0.0044, 0.1413).

Conclusion: This dissertation suggests that excess CVD risk may be detectable among women with a history of LBW at reproductive age. The potential mechanisms might involve vascular dysfunction, inflammation, adiposity, and alterations in lipids.

Public health significance: Women with a history of LBW should be encouraged to optimize their lifestyle in order to prevent future CVD and to evaluate and monitor cardiovascular risk factors (hypertension, obesity, inflammation, and dyslipidemia).

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1.0 INTRODUCTION

1.1 EPIDEMIOLOGY AND POTENTIAL PATHWAYS TO LOW-BIRTH-WEIGHT

An infant's birthweight is an indicator of how pregnancy has progressed. Low birth weight (LBW), defined as birthweight less than 2,500 grams [1], occurred in 8.0% of live births in the United States in 2013 [2]. Birthweight-specific infant mortality rises significantly when birthweight is below 2,500 grams [3]. LBW is a major determinant of neonatal mortality, post-neonatal infant mortality, and infant and childhood morbidity [4]. The public health burden of LBW is driven not only by its subsequent mortality and morbidity, but also by its prevalence in both developing and developed countries. The incidence of LBW in the United States has risen steadily since 1984 (6.7%), reached 8.3% in 2006 [5], followed by a slight decline to 8.0% in 2013 [2]. Preterm birth and restricted fetal growth are two antecedents for LBW delivery. A baby is small at birth either because it was born preterm, because it was small for its gestational age, or because of a combination of the two factors [6]. About two-thirds of LBW infants were born preterm [7], and term LBW infants are usually considered to be growth restricted.

1.1.1 Preterm birth

Preterm birth (PTB) is defined as the delivery of an infant before 37 completed weeks of gestation [1]. PTB accounts for 75% of perinatal mortality [4] and is a leading cause of infant

mortality [8]. Surviving preterm infants are at higher risk of long-term cognitive, motor, sensory, and behavioral deficits compared with infants born at term [9,10]. PTB is often categorized based on clinical circumstances. Spontaneous PTB, initiated by either preterm labor (PTL) or spontaneous preterm premature rupture of membranes (PROM), accounts for approximately 70% of all singleton PTBs. The other 30% are medically indicated to prevent or minimize adverse maternal and/or fetal outcomes [11]. Despite substantial clinical and public health efforts devoted to reduce the incidence of PTB, rates increased by more than one-third from 9.5% in 1981 to 12.8% in 2006 [5], followed by a slight decline to 11.4% in 2013 [7]. Many, but not all, preterm infants were LBW. In 2013, about 46% of preterm infants were LBW [7]. Disorders related to short gestation and low birth weight are the second leading cause of infant death in the United States, following the infant death caused by congenital malformations, deformation and chromosomal abnormalities [12].

1.1.2 Small-for-gestational-age

Since fetal growth is typically not measurable in population-based data, SGA has been used as a proxy indicator for intrauterine growth restricted (IUGR) in many studies. SGA represents a heterogeneous group in regard to etiology. SGA indicates that the infant is either IUGR, constitutionally small but healthy, or has genetic/chromosomal defects [13]. Infants who are SGA are at elevated risk of adverse outcomes in both the perinatal period [14,15] and adulthood [16]. SGA is usually defined as having estimated fetal weight or birthweight below the 10^{th} percentile of certain reference at a particular gestational week [17]. In clinical and epidemiologic studies, population-based, ultrasound-based, and individualized references have been used. For nearly half a century, population-based birthweight references established on the basis of infants

born at various gestational weeks have been used [18]. A population-based reference is usually established with a large sample size study population, including both high-risk and low-risk, both normal and abnormal pregnancy outcomes. However, because neonates born preterm are more likely to be growth restricted, the 10th percentile of preterm infants' birthweight is substantially lower than the 10th percentile of the ultrasound-based fetal weight at the same given gestational week [19,20]. Therefore, using this population-based reference could lead to under-diagnosis of SGA in preterm births. From this point of view, ultrasound-based estimated references of fetal weight are a better choice. Ultrasound-based references were mostly developed in women from European countries, predominantly in white women [21-23]. This might limit its generalizability to other racial/ethnic groups. To develop a method that could identify individual fetus' growth potential, Gardosi et al. created a customized birthweight reference that adjusts for ethnic group, parity, sex of the infant, and maternal height and weight. Recognizing the limitations with previous references, researchers have developed a global reference that adjusted for country or ethnic origin and could be readily adapted to local population [24]. Study indicated that the newly developed reference has improved ability to identify abnormal fetal growth associated with an excess risk of infant death than previously commonly used birthweight references [25].

1.1.3 Potential pathways and risk factors to preterm birth and small-for-gestational-age delivery

PTB and SGA are complications with different etiologic determinants [26,27]. SGA encompasses an extremely heterogeneous group. It has been associated with fetal, placental, and maternal factors. The most common cause of SGA is chronic maternal vascular disorder due to

hypertension, diabetes, and preeclampsia [28,29]. The most pronounced effect is observed among women with early onset hypertension, or chronic hypertension with superimposed preeclampsia [30]. PTB is also a complex outcome initiated by many distinct etiological and pathophysiological pathways [27]. Commonly recognized etiologies and pathways leading to spontaneous PTB and SGA include:

Infection and/or inflammation: out of the suspected PTB pathways, infection and/or inflammation is the only pathological process for which a causal link with PTB, especially early PTB, is established [31,32]. Infections linked to PTB include intrauterine infections, lower genital tract infections, systemic maternal infections, urinary tract infections, bacterial vaginosis, and periodontal disease [33]. Intrauterine infection is one of the most important causes of early PTB and is thought to be responsible for up to 50% of extreme preterm births (infants born before 28 weeks of gestation). Microorganisms are recognized by pattern-recognition receptors, which stimulate the release of inflammatory chemokines and cytokines. The pro-inflammatory cytokines in turn increase the production of prostaglandins and matrix-degrading enzymes, resulting in early labor [34].

Uteroplacental hemorrhage: vascular lesions of the placenta are associated with PTB [35]. The lesions are characterized as failure of physiologic transformation of the spiral arteries, atherosis, and maternal or fetal arterial thrombosis. The mechanism linking vascular lesions of placenta to PTB is thought to be related to utero-placental ischemia.

Stress: stress results in preterm activation of the maternal or fetal hypothalamic-pituitary-adrenal axis and is increasingly recognized as an important cause of later PTB. In vitro and in vivo studies have demonstrated a correlation between hypothalamic corticotropin release, maternal stress, and the timing of birth [33].

Abnormal placental function: placenta transport capacity is a major determinant of nutrient transfer from mother to fetus. Normal placental development involves expansion of fetal villous trophoblast placental surface area and increased concentration of transport protein. Impaired placental vessel development, which may result from reduction in utero-placental blood flow, abnormal villous structure at the interface between maternal and fetal circulation, or abnormality in the umbilical-placental perfusion, leads to restricted fetal growth and perhaps a subset of PTB [36].

Inadequate maternal supply of oxygen and/or nutrients: Normal fetal growth relies upon sufficient maternal supply of oxygen and nutrients. Occlusion of the spiral arteries, resulting from shallow invasion by fetal trophoblasts in maternal spiral arteries, is observed in preeclampsia cases [37]. Products released by the ischemic placenta cause endothelial activation, which may lead to fetal growth restriction as well as a subset of PTB.

Decreased ability of the fetus to use the supply: Two important stages of fetal growth are cellular hyperplasia and cellular hypertrophy. Chromosomal anomalies, genetic conditions, and congenital malformations can lead to cellular hypoplasia, which will restrict fetal growth.

One study has compared risk factors for PTB and SGA births using a national sample of Canadian women [38]. Risk profiles for PTB and SGA share some similarities while have differences (Figure 1). The common risk factors include low weight gain during pregnancy, short statue, primiparity, and high perceived stress. Risk factors unique to PTB include previous PTB, low education, previous medical conditions, new medical condition during pregnancy, and three or more stressful life events 12 months before baby born. Risk factors unique to SGA are smoking during pregnancy, low pre-pregnancy body mass index, recent immigrant status, and young maternal age.

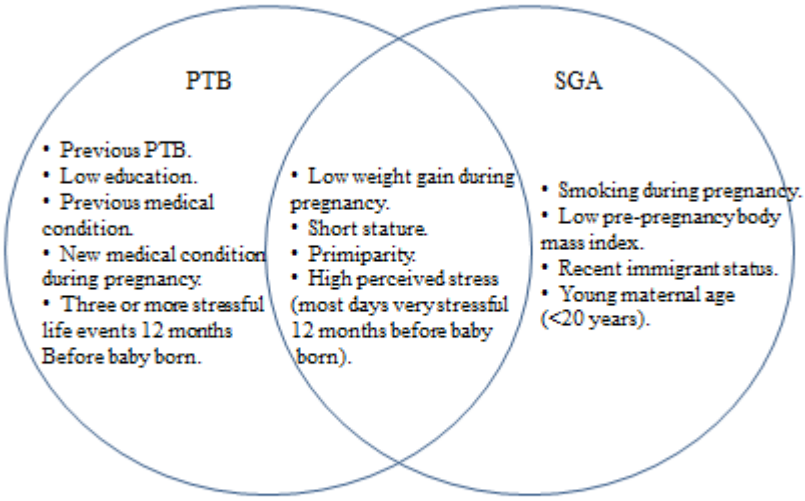

Figure 1. Risk factors for preterm birth and small-for-gestational-age births among Canadian women.

Summary of 1.1: Birth weight is widely used as an indicator of how pregnancy has progressed. Birth weight is determined by two processes: duration of gestation and fetal growth. LBW and its antecedents, SGA and PTB, are pregnancy outcomes that lead to adverse health consequences during infancy, childhood, and adulthood. The two antecedents of LBW have different etiologic determinant, so it is necessary for studies to investigate into Preterm-LBW and SGA-LBW groups separately to gain insights in the etiologic pathways.

1.2 EPIDEMIOLOGY AND RISK FACTORS OF CARDIOVASCULAR DISEASES IN WOMEN

1.2.1 Epidemiology of cardiovascular diseases in women

Cardiovascular diseases (CVD) are the leading cause of mortality in women in the United States [39,40] and constitutes 17% of overall national health expenditure [41,42]. In women, CVD mortality modestly increased until 2000, and then experienced decline [43]. In 2010, the number of deaths in women attributed to CVD was approximately 400,332 in United States [44]. Despite noted reductions in mortality from CVD due to better treatment of CVD in women, this has resulted in more women living with CVD and its potential consequences. The racial/ethnic-disparity in CVD persists for reasons remaining unknown. Cardiovascular death was greatest in black women (566 per 100,000), followed by white women (419 per 100,000) and Hispanic women (332 per 100,000) [45].

1.2.2 Risk factors of cardiovascular diseases

CVD is preventable through appropriate modification and management of risk factors. Identifying and modifying CVD risk factors has become a focus of the American Heart Association in order to reduce the burden of CVD in the United States [46]. In the United States, mortality attributable to CVD has declined. About half of the decline was due to changes in lifestyle risk factors [47]. Lifestyle factors, including cigarette smoking, a diet high in saturated fats, and physical inactivity, have an important causal role in the incidence of CVD. Other established risk factors include hypertension, elevated cholesterol levels, and genetic and

environmental factors. Modifying the common risk factors through lifestyle changes or evidence-based medical therapies has decreased death associated with CVD in women by 23% since 2000 [48]. Menopause is an important CVD risk factor unique to women. CVD is uncommon in premenopausal women, especially among non-smokers. Loss of ovarian function is associated with adverse metabolic changes and increased incidence of CVD. Below is a brief review of several key CVD risk factors, and how they may be uniquely related to CVD risk in women.

Elevated cholesterol levels: optimal cholesterol levels among healthy women are defined by low-density lipoprotein cholesterol (LDL cholesterol) < 100 mg/dL, high-density lipoprotein cholesterol (HDL cholesterol) > 50 mg/dL, and triglycerides < 150 mg/dL [45]. Elevated total cholesterol and LDL levels are major risk factors for CHD in women [49,50]. Before the fifth decades in life, the total cholesterol and LDL cholesterol in women are similar or lower than in men [51]. However, total cholesterol and LDL levels in women rise or even exceed the levels in men following the menopause [52]. HDL cholesterol concentrations are higher in women than in men since young adulthood [53-55]. Similar to LDL cholesterol, the loss of protection from HDL is considered to be a major factor for the increased coronary risk in postmenopausal women [56]. Increase of triglycerides concentrations is a risk factor for CVD, independent of HDL cholesterol in women [57]. The prevalence triglycerides≥ 150 mg/dL among United States women ≥20 years of age has increased from 24.6% (1988-1994) to 29.9% (1999-2000) with stabilization at 26.8% (1999-2008). The prevalence is highest among Mexican American women, followed by non-Hispanic white women, and lowest among non-Hispanic black women [58]. The first-line approach to achieving the optimal cholesterol concentrations is through lifestyle modification [45].

Hypertension: hypertension, defined as systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg or current use of anti-hypertensive medication, is an independent risk factor for ischemic heart disease and stroke [59]. Hypertension is present in approximately one of three adults [60] and contributes to nearly half of CVD-related deaths in the United States [61]. Age-adjusted prevalence of hypertension is significantly higher in black women than in white women. It has been suggested that the improved hypertension control efforts substantially contributed to the decreased death from stroke [62].

Tobacco use: tobacco use is a major modifiable risk factor for CVD in women in the United States [63]. Smoking is known to increase inflammation, thrombosis, and cause endothelial damage and platelet aggregation. Studies showed that smoking appears to be a stronger risk factor for myocardial infarction in middle-aged women than in men [64,65]. Randomized clinical trials have suggested that smoking cessation reduced CVD risk substantially. Although tobacco use has substantially declined in the United States, still, one third of coronary heart disease deaths are attributable to smoking and exposure to secondhand smoke [62].

Obesity**:** obesity is an independent risk factor for CVD in women [66]. It is predicted that obesity will surpass cigarette smoking as the most common CVD risk factor [67]. Overweight and obesity also predispose individuals at increased risk of most major CVD risk factors, such as physical inactivity, diabetes, and hypertension [68]. It is increasingly recognized that body fat distribution is more important than overall excess adiposity in driving CVD risk [69]. Abdominal obesity, in particular visceral fat, is also implicated in metabolic abnormalities that increase CVD risk [70,71]. Chronic accumulation of excess body fat leads to a variety of metabolic changes, increasing the prevalence of CVD risk factors but also affecting systems modulating

inflammation [72]. Obesity rates show a marked variation by race or ethnicity. Non-Hispanic black women have the highest age-adjusted rates of obesity (56.6%) followed by Hispanics (44.4%), non-Hispanic whites (32.8%), and non-Hispanic Asians (11.4%) [73].

1.3 EVIDENCE AND POTENTIAL MECHANISMS OF THE ASSOCIATION BETWEEN LOW-BIRTH-WEIGHT DELIVERY AND MATERNAL CARDIOVASCULAR DISEASES

1.3.1 Evidence for the association between low-birth-weight delivery and maternal cardiovascular diseases

The normal physiological response to pregnancy involves a transient excursion into a metabolic syndrome, which is composed of insulin resistance, hyperlipidemia, and an increase in coagulation factors [74]. Such responses may be considered as “stress” tests on maternal vascular and lipid function [75]. In addition, normal pregnancy also involves up-regulation of the inflammatory cascade [76]. Therefore, adverse pregnancy outcomes, such as LBW, PTB, and SGA, may reflect women’s impaired ability to respond to these metabolic and vascular changes, thus mark women at increased risk of CVD later in life [77].

Increasing studies suggest that a LBW delivery might be a sign of CVD susceptibility in women. Women who delivered a LBW infant are at increased risk of CVD hospitalization or mortality later in life [78-86]. Women with a history of LBW are at two- to three-fold greater risk of CVD [81,82,87]. Birth weight reflects gestational age and fetal growth. Previous studies suggest that mothers of preterm infants are at increased risk of later CVD after accounting for

socio-economic factors, cigarette smoking, and pregnancy-related complications [78-82,85,86]. A register-based study showed that the risk of maternal CVD increased with decreasing gestational age [85]. Compared with women who had a term non-SGA delivery, the hazard ratios of CVD hospitalization or mortality were 1.39 (95% confidence interval 1.22-1.58) and 2.57 (95% confidence interval 1.97-3.34) among women who had moderately and very preterm infants, respectively [85].

1.3.2 Potential mechanisms for the association between preterm birth and maternal cardiovascular diseases

1.3.2.1 Common risk factors shared by preterm birth and cardiovascular diseases

Race disparity: African-American women (hereafter referred to as black) are about twice as likely as non-Hispanic white women (hereafter referred to as white) to have a PTB infant. It is estimated that 54% of the black-white disparity in infant mortality is attributable to the higher incidence of PTB among black women [88]. The racial disparity in CVD between white and black women is also striking. Blacks have the highest overall CVD mortality rates and the highest out-of-hospital coronary death rates than other ethnic groups in the United States [89-91]. Given the higher risk of PTB and CVD in blacks, it is possible that genetic, socioeconomic, or life-style factors contribute to the associations between PTB and maternal CVD risk later in life.

Socioeconomic factors: factors early in a mother's life have a significant impact on both her pregnancy outcomes and CVD risk. Collins and colleagues reported that the social class of neighborhood in which a pregnant woman grew up was strongly associated with her probability of having a LBW infant [92]. Socioeconomic status has been shown to be associated with CVD and CVD risk factors, such as hypertension, obesity, and diabetes. Meanwhile, Miller and

colleagues reported that individuals who grew up in low social class circumstances demonstrated increased pro-inflammatory signaling in their adult life [93]. The up-regulation of inflammatory responses might contribute to the increased risk of LBW delivery and CVD in later life.

Metabolic and vascular factors: metabolic and vascular dysfunction before or during pregnancy appear to predispose women at increased LBW risk. Studies have reported increased risk of delivering preterm or SGA infants among women with metabolic dysfunction. Pre-pregnancy dyslipidemia was related to subsequent PTB risk [94]. There is also evidence of a significant inverse association between diastolic blood pressure and birth weight in women who had hypertension during pregnancy [95]. The metabolic and vascular risk factors existing before or during pregnancy may explain why women who delivered preterm or SGA infants are at higher risk of developing CVD.

1.3.2.2 Inflammation

Infection and/or inflammation is a well-established causal pathway to PTB. Serum C-reactive protein (CRP) [96-98] and interleukin-6 (IL-6) [31] during pregnancy are linked to spontaneous PTB, which accounts for about 70% of all singleton PTB [31]. The other 30% are medically indicated PTB with preeclampsia as one of the leading causes [11,99]. Preeclampsia is also associated with higher CRP and IL-6 concentrations during [100,101] and after [102] pregnancy. Recently, one retrospective cohort study suggested that women who experienced PTB, specifically medically indicated PTB, had increased CRP concentrations in later life compared to women who delivered at term [103].

Numerous studies have identified low-grade inflammation as a key process in atherosclerosis and CVD [104]. Chronically higher concentrations of CRP, a sensitive marker of inflammation regardless of etiology, add additional information to predict future cardiovascular

events, after initial screening with conventional risk factors alone [105-108]. Studies suggest that CRP is more strongly related to CVD risk in women than men [109,110]. The Reynolds Risk Score incorporating CRP has been proposed for cardiovascular risk assessment in women [111]. Therefore, the associations between PTB and CVD might relate to up-regulation of inflammation. Women with a pro-inflammatory phenotype may be at greater risk of developing up-regulation of inflammation during pregnancy, which would put them at higher risk of PTB. Meanwhile, as inflammation is an independent predictor of CVD in women, these women may be also more likely to develop CVD later in life.

1.3.3 Potential mechanisms for the association between small-for-gestational-age delivery and maternal cardiovascular diseases

Vascular dysfunction: normal pregnancy is characterized by physiological changes to meet the mother's own metabolic needs, to facilitate placental circulation, and to provide the fetus with adequate oxygen and nutrient supply [74]. The changes include increase in blood volume [112], increase in heart rate [113], and reduction in systemic vascular resistance [114]. Apparently healthy women with underlying CVD risk may have impaired ability to adjust to this hemodynamic challenge. This could result in placental dysfunction, thus leading to SGA [36]. It is documented that reduced blood volume expansion was associated with LBW and intrauterine growth restriction [115].

Preeclampsia: preeclampsia is a pregnancy complication characterized by high blood pressure with proteinuria that begins after 20 weeks of gestation in a woman whose blood pressure had been normal [116]. Preeclampsia is one of the most common causes of maternal and fetal mortality and morbidity [117,118]. It is one major causal factor for growth restriction.

Some of the risk factors for preeclampsia are also predictive of cardiovascular disease later in life [75,119]. Women with a history of preeclampsia demonstrate hypertension, higher fasting insulin, lipid, coagulation factors, and defect in endothelial-dependent vascular function in later life [120-123]. Bonamy et al. proposed that "preeclampsia might be the tip of the iceberg of subclinical defects in placentation, characterized by endothelial dysfunction and systemic inflammation, related to preterm birth, intrauterine growth restriction, stillbirth, and CVD risk in the mothers" [85]. This is further supported by an investigation of mothers in the Avon Longitudinal Study of Parents and Children. Women with a prior PTB had higher blood pressure 18 years after delivery. The association was largely explained by hypertensive disorders during pregnancy [124]. Based on the above evidence, factors existing prior to pregnancy may contribute to occurrence of preeclampsia. After delivery, as the women age, the same factors could contribute to increased risk of metabolic and vascular diseases.

1.3.4 Limitations of existing studies

The existing literature suggested several gaps in knowledge related to the association between delivery of a LBW infant and maternal CVD risk later in life. Most of prior studies on LBW and maternal CVD risk were registry-based studies. Those studies considered hospitalization or death from coronary heart disease, cerebrovascular events, or heart failure as main outcomes. Individuals' measurements of modifiable risk factors (such as, hypertension and obesity) were usually not collected in those studies. Understanding the associations between LBW delivery and modifiable maternal CVD risk factors will be of more practical use to identify individuals at CVD risk at an early enough stage to benefit from effective interventions.

In addition, some existing studies did not account for the potential confounding factors (such as, body mass index, cigarette smoking during pregnancy) that are associated with PTB, SGA, and maternal CVD risk later in life. There is evidence that adjusting for smoking attenuated the association between LBW for gestational age and later maternal CVD risk. Conducting a study which collects the information on cigarette smoking and other potential confounding factors will help rule out the possible confounding effects.

Moreover, it is not known whether the maternal CVD risk increase differs with a history of PTB-LBW vs. SGA-LBW. PTB and SGA may involve different etiologic pathways leading to later-onset maternal CVD. Therefore, a mixture of PTB and SGA is not very informative when maternal CVD risk, the outcome of interest, may differentially relate to prematurity or growth restriction. Further, by investigating PTB-LBW and SGA-LBW separately, we are able to gain insights into possible different mechanisms leading to prematurity or growth restriction.

At last, previous studies were unable to explore the race/ethnicity-specific associations between LBW and maternal CVD risk due to their homogenous racial/ethnic group. It is well established that African Americans are at higher risk of PTB, SGA, and CVD risk. Therefore, race is possible an important effect modifier for the associations.

Summary of 1.3: Epidemiologic evidence suggests that women who delivered a preterm or SGA infant are at higher CVD risk later in life. Maternal genotypes and phenotypes associated with CVD risk may impair these women's ability to adjust to the vascular challenge during pregnancy, thus presenting as PTB or SGA during pregnancy. The same factors will also contribute to the increased CVD risk in mothers and infants later-in-life.

1.4 OBJECTIVES AND ORGANIZATION OF THE DISSERTATION

The objective of this dissertation is to provide insights on possible underlying pathways linking a prior LBW delivery to long term maternal cardiovascular health. This dissertation is presented in a multiple manuscript format. Chapter 2, 3, and 4 are written as individual research papers. The specific tasks include:

(1) To evaluate blood pressure and hypertension in women who reported a prior preterm or SGA-LBW delivery in the National Health and Nutrition Examination Survey 1999-2006. To explore if race/ethnicity, menopause status, and years since last pregnancy modified the above associations

(2) To examine associations between PTB and maternal interleukin-6 and C-reactive protein concentrations eight years after delivery in the Women and Infant Study of Healthy Hearts study.

(3) To examine the relationship between a prior PTB and adiposity measures eight years after delivery in a subgroup from the Women and Infant Study of Healthy Hearts study. To investigate how adiposity measures may contribute to the association between a prior preterm birth and subsequent cardiometabolic and inflammatory risk factors.

Chapter 5 draws the conclusions, summarizes the original contributions of the dissertation, and proposes several topics for future research. Chapter 6 discusses public health significance.

2.0 MATERNAL HYPERTENSION AFTER A LOW-BIRTH-WEIGHT DELIVERY DIFFERS BY RACE/ETHNICITY: EVIDENCE FROM THE NATIONAL HEALTH AND NUTRITION EXAMINATION SURVEY (NHANES) 1999-2006 *

ABSTRACT

Studies have suggested an increase in maternal morbidity and mortality due to cardiovascular diseases in women with a prior low-birth-weight (LBW, <2,500 grams) delivery. This study evaluated blood pressure and hypertension in women who reported a prior preterm or small-for-gestational-age (SGA) LBW delivery in the National Health and Nutrition Examination Survey 1999-2006 (n=6,307). This study also aimed to explore if race/ethnicity, menopause status, and years since last pregnancy modified the above associations.

A total of 3,239 white, 1,350 black, and 1,718 Hispanics were assessed. Linear regression models were used to evaluate blood pressure by birth characteristics (preterm-LBW, SGA-LBW,

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and birthweight≥2,500). Logistic regression models estimated the odds ratios (OR) of hypertension among women who reported a preterm-LBW or SGA-LBW delivery compared with women who reported an infant with birthweight≥2,500 at delivery. Overall, there was a positive association between a preterm-LBW and hypertension (adjusted OR=1.39, 95% confidence interval (CI) 1.02-1.90). Prior SGA-LBW also increased the odds of hypertension, but the estimate did not reach statistical significance (adjusted OR=1.21, 95% CI 0.76-1.92). Race/ethnicity modified the above associations. Only black women had increased risk of hypertension following SGA-LBW delivery (adjusted OR=2.09, 95% CI 1.12-3.90). Black women were at marginally increased risk of hypertension after delivery of a preterm-LBW (adjusted OR=1.49, 95% CI 0.93-2.38). Whites and Hispanics had increased, but not statistically significant, risk of hypertension after a preterm-LBW (whites: adjusted OR=1.39, 95% CI 0.92-2.10; Hispanics: adjusted OR=1.22, 95% CI 0.62-2.38). Stratified analysis indicated that the associations were stronger among premenopausal women and women with a shorter duration after last pregnancy. The current study suggests that in a representative United States population, women with a history of preterm- or SGA-LBW deliveries have increased odds of hypertension and this risk appears to be higher for black women and younger women.

2.1 INTRODUCTION

Women who have delivered a low-birth-weight (LBW) infant (birth weight less than 2,500 grams) are at increased risk for subsequent incidence and mortality from cardiovascular diseases

(CVD) [78,79,81,82]. LBW occurred in 8.1% of the live births in the United States in 2011 [125]. About two-thirds of LBW infants are born preterm (PTB, delivery of an infant before 37 completed weeks of gestation), and the other LBW infants are considered to be small-for-gestational-age (SGA, most commonly defined as a fetal weight or birth weight below the 10^{th} percentile at a particular gestational week) [126]. PTB and SGA, the two antecedents of LBW [6], have distinct etiologies [26,28,127]. PTB complicates 6% to 12% of deliveries in developed countries [128]. PTB is often categorized based on clinical circumstances. Spontaneous PTB accounts for approximately two-thirds of all singleton PTBs. Infection and/or inflammation are well-established causal pathways for spontaneous PTB, especially early PTB [31,32]. Medically indicated PTB, which accounts for the other one-third of PTBs, has a dominantly vascular etiology [129]. SGA, which accounts for approximately 5% to 7% of deliveries, is also primarily a vascular-related disorder [28,29].

A recent study with the National Health and Nutrition Examination Survey (NHANES) 1999-2006 data suggested that giving birth to a SGA infant is strongly and independently associated with maternal ischemic heart disease [130]. Increasing evidence indicates that PTB or SGA delivery and later maternal CVD risk share some common features such as vascular endothelial dysfunction, metabolic syndrome, hypertension, and dyslipidemia [131,132].

Hypertension is a major cardiovascular risk factor, contributing to approximately half of all CVD-related deaths [61]. Pregnancy has been viewed as a cardiovascular “stress test” for women [128]. A registry-based study indicated that women with a prior PTB had higher risk of hypertension after excluding preeclampsia cases [132]. Recently, a cohort study of 679 women suggested that women with a prior PTB had higher blood pressure eight years after the delivery [131]. In addition, hypertension before or during pregnancy increases the risk of preterm or SGA

birth [133,134]. Taken together, the evidence supports the existence of common predisposing risk factors for both LBW and hypertension. The pre-pregnancy subclinical and clinical vascular aberrations that contribute to LBW may persist after pregnancy and increase the mother's CVD risk in their later life.

Previous studies relating LBW to maternal risk of elevated blood pressure later in life were conducted in predominantly white women [131,132]. Therefore, those studies were not able to investigate the race/ethnicity-specific associations. Racial/ethnic differences in PTB, SGA, and hypertension persist despite substantial clinical and public health efforts [88,135]. Hypertension is particularly prevalent and poorly controlled in blacks compared with whites [135,136]. The prevalence of hypertension in Hispanic populations is similar to white populations; however, blood pressure control in Hispanics is not as successful as in whites [135]. It is also well acknowledged that black women experience much higher rates of PTB, SGA, and LBW than any other ethnic group in the United States [126]. Hispanic women have slightly higher PTB rates and similar LBW rates compared to white women [6,99]. However, to date, the interrelationship among race/ethnicity, LBW delivery, and subsequent maternal blood pressure has not been evaluated.

This study sought to examine the independent associations between the two antecedents of LBW (PTB and SGA) and subsequent maternal blood pressure and hypertension in a representative United States population. This study also investigated whether the associations between PTB or SGA and subsequent maternal blood pressure and hypertension vary by race/ethnicity. Moreover, to explore the extent to which hypertension risks associated with pregnancy complications change over time, this study also stratified the population by menopausal status and time since last pregnancy. The hypothesis is that women with a prior PTB

or SGA delivery will have increased odds of hypertension, and this will be more pronounced in black compared to white women.

2.2 MATERIALS AND METHODS

2.2.1 Data source and study population

This study used data from the NHANES (http://www.cdc.gov/nchs/nhanes.htm) 1999-2006. NHANES are public use data files without identifiers, released by the National Center for Health Statistics. They are exempt from Institutional Review Board review under category 4 from 45 Code of Federal Regulations Part 46. NHANES is an ongoing survey on health and nutritional status designed to be nationally representative of the non-institutionalized, United States population [137]. Since 1999, NHANES has conducted a continuous annual survey using a stratified multi-stage probability design to obtain nationally representative samples, with an oversample of low-income individuals, individuals between 12 and 19 years of age, adults over the age of 60 years, blacks, and Mexican Americans. NHANES data are collected in two phases. First, the participants' health history, health behaviors, and risk factors are obtained during a home interview. Participants are then invited to take part in a medical examination where they receive a detailed physical and laboratory examination.

Of the 21,210 female participants enrolled in NHANES 1999-2006, women who were younger than 20 years of age (n=10,509, 50%), women who were pregnant at interview (n=1,173, 6%), women who did not complete the interview and examination (n=662, 3%), women who did not report previous live birth delivery (n=2,308, 11%), and women who reported

race/ethnicity other than white, black or Hispanic (n=209, 1%), were excluded in consecutive steps. Women who did not answer the pregnancy history questions were also excluded (n=42, 0.2%) as these questions were used to construct the main exposure variable (see below). Thus, a total of 6,307 (30%) women were included in the analysis. Among these women, 3,239 (51%) women were non-Hispanic white (white), 1,350 (22%) women were non-Hispanic black (black), and 1,718 (27%) women were Mexican American and other Hispanic (Hispanic). (Figure 2).

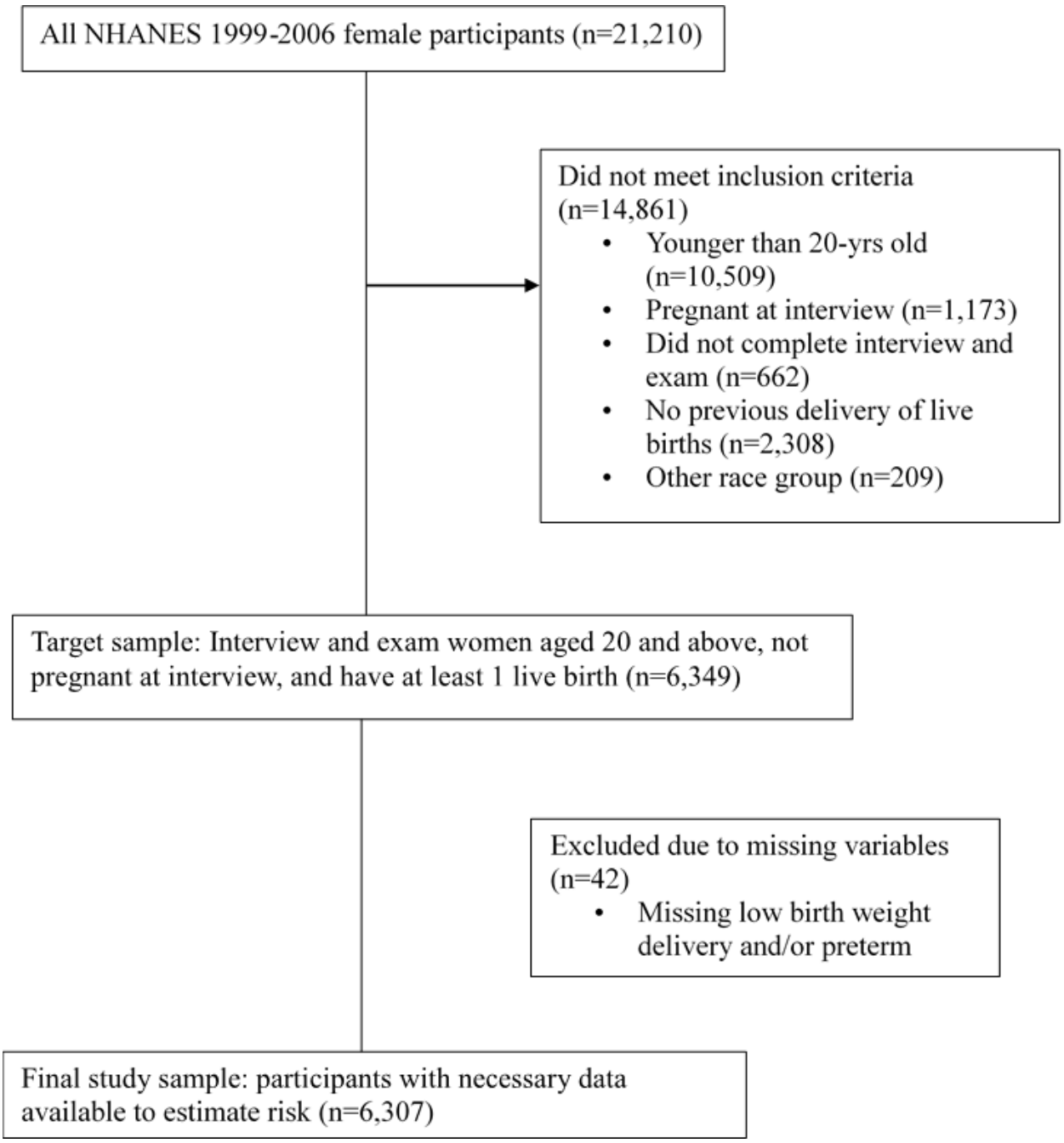

Figure 2. Participants flowchart.

2.2.2 Study variables

Outcomes-blood pressure and chronic hypertension

The primary outcomes of interest were blood pressure (systolic, SBP and diastolic, DBP) and chronic hypertension. NHANES measured up to four blood pressures (BP), and the averages of these were calculated. Chronic hypertension was defined as self-report use of anti-hypertensive medication, or SBP≥140 mm Hg or DBP≥90 mm Hg at the NHANES interview.

Exposure-birth characteristics

The exposure variable of interest was created to represent participants' response to two reproductive history questions. The participants were first asked "Did any child weigh less than 5.5 pounds (2,500 grams) at birth?" Women who answered yes to this LBW question were asked "How many of these babies were born preterm?" A three-level, mutually exclusive categorical variable (preterm-LBW, term-LBW, and birth weight of ≥2,500 grams) was created from these two questions to represent the birth characteristics. According to Hadlock's study, infants born at term and with BW<2,500 grams(g) were below the 10th percentile in the United States [138]. SGA is typically defined as birth weight (BW) below the 10th percentile for gestational age. Therefore, the term-LBW group was referred to as SGA-LBW group in this study. Among 565 women in the preterm-LBW group, 106 (18.8%) had more than one preterm delivery, which were too few to be evaluated separately.

Covariates

Demographic, health-related, and reproductive characteristics were considered as potential confounding variables. Demographic variables included age (in years), education (less than high school graduate, high school graduate or above), and income (less than $20,000, $20,000-$45,000, more than $45,000). Health-related characteristics included insurance (having

insurance/none), body mass index (BMI, the *weight* in *kilograms* divided by the square of the height in meters), waist circumference (cm), current tobacco use (yes, no), current alcohol use (yes, no), fiber in diet (gram/day), sodium intake (mg/day), physical inactivity (yes, no), family history of ischemic heart disease (yes, no), family history of diabetes (yes, no), family history of hypertension/stroke (yes, no), diabetes status (fasting blood glucose ≥126 mg/dl and/or current use of insulin or diabetes medications), and anti-hypertensive medication use (only for analysis with blood pressure as outcome). Reproductive characteristics included the number of live births, years since last pregnancy, and menopause status. Women were considered to be post-menopause if they answered "menopause" to the question "What is the reason that you have not had a period in the past 12 months?"

2.2.3 Statistical analysis

This study combined the NHANES 1999-2006 data, therefore 8-year interview/medical exam sampling weight variables were created and incorporated into the analysis to account for the NHANES sampling schemes. For continuous maternal characteristics, the race/ethnicity-specific differences in means between PTB-LBW, SGA-LBW, and BW≥2,500 g groups were evaluated using univariate weighted linear regression. For categorical variables, the differences in proportions were evaluated with Rao-Scott Chi-Square test [139]. Maternal characteristics that were significantly different among three birth characteristics groups were considered as potential confounders in the multivariable analysis. To estimate the age-adjusted prevalence of hypertension according to race/ethnicity, the population was standardized to the 2000 United States Census population with three age groups: 20-39, 40-59, and 60 and above, as recommended by National Center for Health Statistics [140].

Linear regression models were used to evaluate SBP and DBP according to LBW history, with adjustment for age at interview, race, education, insurance, cigarette smoking, waist circumference, fiber intake, sodium intake, the number of live births, years since last pregnancy, menopause status, family history of heart attack and diabetes, and anti-hypertensive medication use. Effect modification on the additive scale by race/ethnicity, menopausal status, and years since last pregnancy were assessed in the full model with potential confounders. T-tests were used to test the significance of regression coefficient of the interaction terms. If statistically significant interactions were found, stratified analyses were then performed. Sensitivity analyses were conducted among the women who were not taking anti-hypertensive medication at NHANES interview.

Logistic regression models were then developed to estimate the odds ratio (OR) of hypertension among women with a prior Preterm-LBW or SGA-LBW delivery, compared with women with a BW≥2,500 g delivery. Effect modification on the multiplicative scale by race/ethnicity, menopausal status, and years since last pregnancy was assessed using likelihood ratio tests (α=0.05) in the full model adjusted for potential confounders. If statistically significant interactions were found, stratified analyses were then performed. In the multivariable analyses, potential confounders were the same covariates as above except anti-hypertensive medication use as this was included in the construction of the outcome.

An important unmeasured covariate in the dataset was pre-pregnancy BMI. Pre-pregnancy underweight is associated with LBW, and pre-pregnancy obesity is a risk factor for preeclampsia which is associated with maternal hypertension. In the absence of pre-pregnancy BMI data, models were additionally adjusted for BMI at age 25 as a proxy for this potential

confounder. All p-values were two-sided and were considered statistically significant if less than 0.05. Statistical analyses were done using SAS 9.3 (SAS Institute Inc., Cary, NC).

2.3 RESULTS

Table 1 displays the race/ethnicity-specific maternal characteristics by Preterm-LBW, SGA-LBW, and BW≥2,500 g groups. The percentage of women with at least one LBW infant was highest in blacks (17.9%), followed by Hispanics (13.2%), and whites (11.8%). At the NHANES visit, black or Hispanic women were younger, had higher mean BMI and waist circumferences, had more disadvantaged socioeconomic status profiles, and were more likely to have a family history of diabetes, compared with white women. Hispanics were less likely to be insured than whites or blacks. As for clinical characteristics, compared with whites, blacks had more anti-hypertensive medication use, whereas Hispanics had less anti-hypertensive medication use.

Table 1. Unadjusted race/ethnicity-specific maternal characteristics of the study population: National Health and Nutrition Examination Surveys 1999-2006 (n=6,307).

	White, non-Hispanic (n=3,239)			Black, non-Hispanic (n=1,350)			Hispanic (n=1,718)		
	Preterm	**SGA**	**BW≥2,500g**	**Preterm**	**SGA**	**BW≥2,500g**	**Preterm**	**SGA**	**BW≥2,500g**
	n=257, 8%	n=126, 3%	n=2,856, 89%	n=164, 12%	n=78, 5%	n=1,108, 83%	n=144, 8%	n=83, 4%	n=1,491, 88%
Age, year	51.3±1.0	58.8±1.6	51.7±0.4 *	44.6±1.2	50.0±2.2	46.5±0.5	44.6±2.6	46.0±2.5	44.0±0.6
Education									
Less than high school graduate	21%	28%	14% *	32%	48%	31% *	52%	60%	48%
Household Income									
Less than $20,000	26%	31%	18% *	37%	55%	35%	31%	59%	32% *
$20,000 to $45,000	30%	26%	32%	34%	26%	38%	45%	29%	42%
More than $45,000	44%	43%	50%	29%	19%	27%	24%	12%	26%
Insurance									
No insurance	15%	13%	11%	13%	13%	19%	36%	59%	35% *
Current smoker	33%	28%	22% *	22%	27%	21%	27%	29%	15% *
Current alcohol user	63%	64%	66%	51%	42%	45%	48%	45%	46%
Fiber in diet (g/d)	12.0±0.6	13.3±0.7	13.9±0.2 *	10.7±0.5	10.9±1.1	11.6±0.3	12.3±1.1	14.3±1.0	15.7±0.6 *
Sodium (mg/day)	2.7±0.1	2.7±0.1	2.9±0.1	2.7±0.1	2.4±0.2	2.9±0.1 *	2.8±0.2	2.6±0.3	2.7±0.1
Waist circumference (cm)	90.4±1.0	94.1±1.8	93.9±0.4 *	96.7±1.3	102.8±2.2	99.4±0.5	92.0±1.5	96.7±2.5	94.5±0.5
BMI	27.0±0.5	27.6±0.9	28.1±0.2	30.5±0.7	33.2±0.9	31.8±0.3	28.1±0.7	29.8±1.2	29.4±0.2
Physical inactivity	21%	29%	25%	33%	44%	31%	25%	17%	23%

Table 1 (continued).

Menopause	55%	73%	54% *	40%	55%	44%	35%	48%	36%
Number of live births									
1-2	39%	39%	58% *	43%	39%	56% *	45%	37%	50%
≥3	61%	61%	42%	57%	61%	44%	55%	63%	50%
Years since last pregnancy									
<10 years	24%	7%	23% *	30%	30%	30%	40%	34%	38%
10-25 years	34%	36%	34%	42%	28%	38%	38%	43%	40%
>25 years	42%	57%	43%	28%	42%	32%	22%	23%	22%
Family history									
Ischemic heart disease	22%	19%	19%	14%	29%	13% *	16%	3%	15%
Diabetes	47%	47%	50%	71%	63%	59% *	58%	62%	54%
HTN/stroke	35%	28%	34%	49%	59%	48%	39%	30%	32%
Diabetes	5%	5%	7%	11%	18%	14%	10%	12%	10%
Anti-HTN medication	22%	32%	24%	36%	52%	32% *	20%	10%	14%
BMI at age 25	22.2±0.4	21.4±0.3	22.6±0.1 *	23.8±0.5	24.7±0.7	24.3±0.2	22.8±0.5	23.6±0.6	23.8±0.2

*** p<0.05 for the test of overall differences between Preterm, SGA, and BW≥ 2500g groups within each race/ethnicity group.**
Abbreviation: SGA: small-for-gestational-age; BW: birthweight; BMI: body mass index; HTN: hypertension.

Overall, black women had a higher prevalence of hypertension (44%) compared to white (36%) and Hispanic (24%) women. Within each race/ethnicity group, women who had delivered a SGA-LBW or Preterm-LBW infant had higher prevalence of hypertension than the BW≥2,500 g group. Of note, black women who delivered infants with BW≥2,500 g had a higher prevalence of hypertension compared to any group of white or Hispanic women (Figure 3).

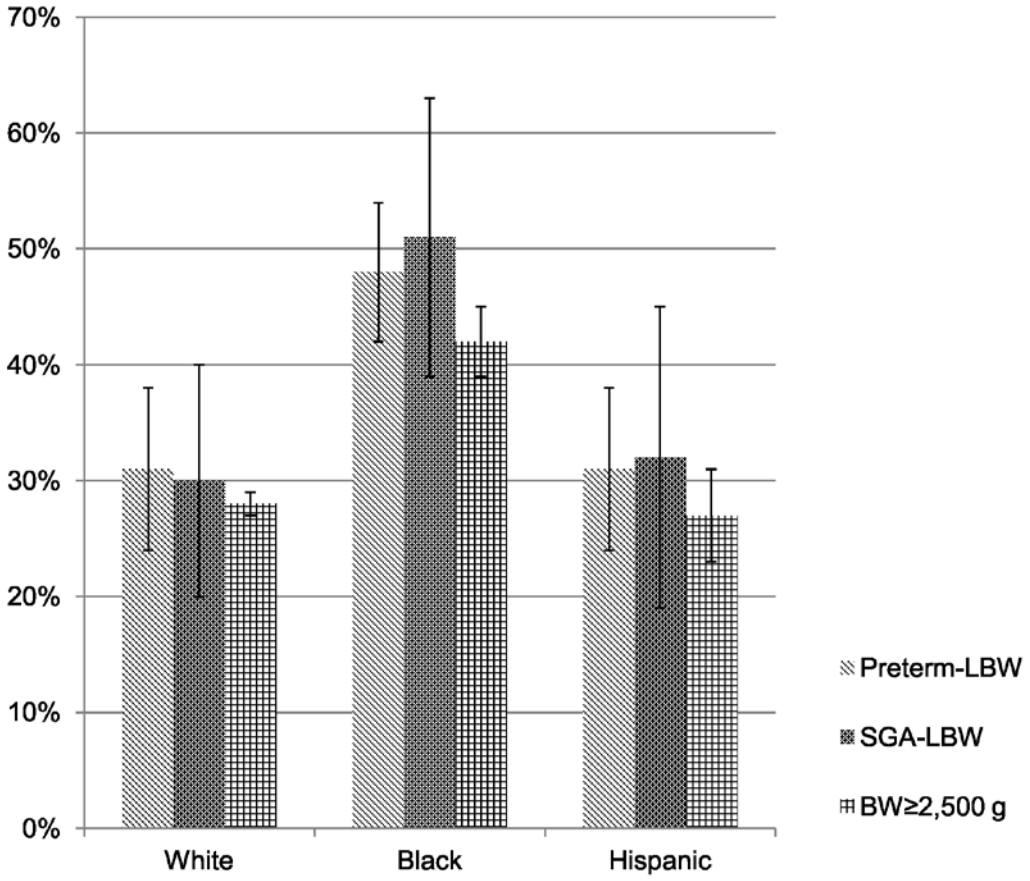

Figure 3. Prevalence of hypertension in white, black, and Hispanic women.

In the total study population, after adjustment for age, race, education, insurance, cigarette smoking, waist circumference, fiber intake, sodium intake, menopause status, the number of live births, family history of heart attack and diabetes, and years since last pregnancy, women with a prior Preterm-LBW delivery had higher odds of hypertension compared with women with a BW≥2,500 g delivery (OR=1.39; 95% confidence interval [CI], 1.02-1.90, Table 2). A prior SGA-LBW was also associated increased the odds of hypertension, but the estimate did not reach statistical significance (OR, 1.21; 95% CI, 0.76-1.92). Assessment of interactions indicated that race/ethnicity, menopause status, and years since last pregnancy each significantly modified the association between pregnancy characteristics and maternal hypertension ($p<0.05$). Women with a prior Preterm-LBW infant had higher mean SBP compared to women with BW≥2,500 g delivery (132.6 mmHg vs. 130.3 mmHg, $p=0.01$), after adjusting for the confounding variables. Women with a prior SGA-LBW delivery also had significantly higher adjusted SBP than women with a BW≥2,500 g delivery (133.3 mmHg vs. 130.3 mmHg, $p=0.04$). There was no significant difference in DBP between the LBW subgroups and BW≥2,500 g delivery group. None of the interactions tested were statistically significant in the linear regression models for SBP or DBP.

Table 2. Adjusted blood pressure and odds ratio of hypertension: National Health and Nutrition Examination Surveys 1999-2006 (n=6,307).

	Preterm (n=565)	**SGA (n=287)**	**BW≥2,500g (n=5,455)**
	Mean±SE	Mean±SE	Mean±SE
SBP (mmHg) §	132.6±0.8 †	133.3±1.5 †	130.3±0.6 *
DBP (mmHg) §	69.9±0.7	68.8±1.2	69.5±0.5
	Odds ratio (95% CI)	Odds ratio (95% CI)	
HTN ‡	**1.39 (1.02-1.90)**	1.21 (0.76-1.92)	reference

*** p<0.05 for the test of overall difference in SBP or DBP between Preterm, SGA, and BW≥2,500g groups.**

† p<0.05 for the test of difference in the pairwise comparison of SBP or DBP between Preterm or SGA and BW≥2,500g.

§ Adjusted for age at interview, race, education, insurance, cigarette smoking, waist circumference, fiber intake, sodium intake, parity, years since last pregnancy, menopause status, family history of heart attack, family history of diabetes, and anti-hypertensive medication use.

‡ Adjusted for age at interview, race, education, insurance, cigarette smoking, waist circumference, fiber intake, sodium intake, parity, years since last pregnancy, menopause status, family history of heart attack, and family history of diabetes.

Abbreviation: SBP: systolic blood pressure; DBP: diastolic blood pressure; HTN: hypertension; SGA: small-for-gestational-age; BW: birthweight; SE: standard error; 95% CI: 95% confidence interval; ref: reference group.

The adjusted ORs of hypertension varied by race/ethnicity groups (Table 3). In black, compared to women with BW >2500 g, the odds of hypertension were marginally higher among those with Preterm-LBW (adjusted OR, 1.49; 95% CI, 0.93-2.38; p-value=0.10) and significantly higher among those with SGA-LBW (adjusted OR, 2.09; 95% CI, 1.12-3.90; p-value=0.02) births. White and Hispanic women also had higher odds of hypertension after a Preterm-LBW (whites: adjusted OR, 1.39; 95% CI, 0.92-2.10; Hispanics: adjusted OR; 1.22, 95% CI, 0.62-2.38) but the estimates did not reach statistical significance. There were no observed associations between hypertension and SGA-LBW delivery among whites (adjusted OR, 1.11; 95% CI, 0.61-2.02) or Hispanics (adjusted OR, 0.86; 95% CI, 0.36-2.05). Additional adjustment for BMI at age 25 as a proxy for pre-pregnancy BMI did not change the results.

The adjusted ORs of hypertension also varied by menopausal status and years since last pregnancy (Table 3). Associations in the pre-menopausal group were similar to what were found in the total population. In pre-menopausal group, increased odds of hypertension were observed among women after a preterm-LBW delivery (adjusted OR, 2.22; 95% CI: 1.32-3.72). A prior SGA-LBW was also associated with increased the odds of hypertension, but the estimate did not reach statistical significance (OR, 1.32; 95% CI, 0.61-2.84). No significant differences in hypertension among preterm-LBW vs. BW >2500 g or among SGA-LBW vs. BW >2500 g were observed in post-menopausal group. The study population was also stratified by years since last pregnancy (<10 years, 10-25 years, and >25 years) to understand if LBW delivery was temporally associated with maternal hypertension. Women with a shorter duration since last pregnancy (<10 years) had increased odds of hypertension after a prior Preterm-LBW (adjusted OR, 2.96; 95% CI: 1.28-6.88) or SGA-LBW (adjusted OR, 2.71; 95% CI: 0.90-8.14). No

significant differences in hypertension were observed among preterm-LBW vs. BW >2500 g or among SGA-LBW vs. BW >2500 g in the 10-25 years and above 25 years groups.

Table 3. Adjusted odds ratio of hypertension by race/ethnicity, menopause status, and years since last pregnancy: National Health and Nutrition Examination Surveys 1999-2006 (n=6,307).

By race/ethnicity (n=6,307)									
	White, non-Hispanic (n=3,239)			**Black, non-Hispanic (n=1,350)**			**Hispanic (n=1,718)**		
	Preterm (n=257)	**SGA (n=126)**	**BW ≥2,500g (n=2,856)**	**Preterm (n=164)**	**SGA (n=78)**	**BW ≥2,500g (n=1,108)**	**Preterm (n=144)**	**SGA (n=83)**	**BW ≥2,500g (n=1,491)**
	Odds ratio (95% CI)	Odds ratio (95% CI)		Odds ratio (95% CI)	Odds ratio (95% CI)		Odds ratio (95% CI)	Odds ratio (95% CI)	
HTN ‡	1.39 (0.92-2.10)	1.11 (0.61-2.02)	reference	1.49 (0.93-2.38)	**2.09 (1.12-3.90)**	reference	1.22 (0.62-2.38)	0.86 (0.36-2.05)	reference
By menopause status (n=6,307)									
	Premenopausal (n=2,679)			**Menopausal (n=3,628)**					
	Preterm (n=91)	**SGA (n=25)**	**BW ≥2,500g (n=1,049)**	**Preterm (n=85)**	**SGA (n=27)**	**BW ≥2,500g (n=522)**			
	Odds ratio (95% CI)	Odds ratio (95% CI)		Odds ratio (95% CI)	Odds ratio (95% CI)				
HTN §	**2.22 (1.32-3.72)**	1.32 (0.61-2.84)	reference	1.08 (0.76-1.53)	1.17 (0.66-2.05)	reference			
By years since last pregnancy (n=6,304)									
	<10 years (n=1,306)			**10-25 years (n=1,793)**			**>25 years (n=2,935)**		
	Preterm (n=251)	**SGA (n=124)**	**BW ≥2,500g (n=2,759)**	**Preterm (n=157)**	**SGA (n=73)**	**BW ≥2,500g (n=1,060)**	**Preterm (n=138)**	**SGA (n=80)**	**BW ≥2,500g (n=1,392)**
	Odds ratio (95% CI)	Odds ratio (95% CI)		Odds ratio (95% CI)	Odds ratio (95% CI)		Odds ratio (95% CI)	Odds ratio (95% CI)	
HTN †	**2.96 (1.28-6.88)**	2.71 (0.90-8.14)	reference	1.36 (0.79-2.32)	1.20 (0.68-2.12)	reference	1.27 (0.86-1.87)	1.06 (0.59-1.91)	reference

‡ Adjusted for age at interview, education, insurance, cigarette smoking, waist circumference, fiber intake, sodium intake, parity, years since last pregnancy, menopause status, family history of heart attack, and family history of diabetes.

§ Adjusted for age at interview, race, education, insurance, cigarette smoking, waist circumference, fiber intake, sodium intake, parity, years since last pregnancy, family history of heart attack, and family history of diabetes.

† Adjusted for age at interview, race, education, insurance, cigarette smoking, waist circumference, fiber intake, sodium intake, parity, menopause status, family history of heart attack, and family history of diabetes.

Abbreviation: HTN: hypertension; SGA: small-for-gestational-age; BW: birthweight; 95% CI: 95% confidence interval; ref: reference group.

2.4 DISCUSSION

To our knowledge, this is the first study to demonstrate race/ethnicity-specific relationships between a LBW delivery and hypertension after pregnancy. Evidence from this United States representative population suggested that race/ethnicity modified the association between a previous LBW delivery and maternal hypertension, such that risk may be higher among black women. These findings were robust to adjustments for measured confounders, such as age, education, cigarette smoking, waist circumference, sodium intake, family history of heart attack, and family history of diabetes. Secondly, this study also assessed the separate effects of PTB and SGA in order to distinguish the contributions of these two determinants of LBW. While the Preterm-LBW association with maternal hypertension was similar regardless of maternal race/ethnicity, the link with SGA-LBW appeared to be limited to black women. Thirdly, in general these associations appeared to be stronger among women with a shorter duration after last pregnancy (<10 years). The findings are consistent with the hypothesis that the link between LBW and later maternal CVD involves vascular dysfunction. This may be particularly important among black women, and these associations may be stronger in younger compared to older women.

The positive association between Preterm-LBW and subsequent maternal hypertension is consistent with previous studies [87,132]. The underlying mechanisms linking PTB and maternal increased odds of hypertension remain unclear. One potential mechanism may be inflammation. It is known that inflammation is causally related to spontaneous PTB, especially early PTB [31]. One study has indicated that many years after delivery, women with a prior preterm versus term

birth had increased C-reactive protein (CRP) levels, a marker of acute and chronic inflammation [103]. Inflammation is also potentially implicated in the development of cardiovascular diseases [141]. It seems plausible that women with a pro-inflammatory status during pregnancy delivered preterm infants, and after delivery this pro-inflammatory status may persist and relate to arterial stiffness, subclinical cardiovascular diseases, and hypertension later in life. Alternatively, preeclampsia is associated with preterm delivery and later life maternal risk of hypertension and may explain the associations we detected. NHANES did not collect information on preeclampsia, however, adjustment for preeclampsia has not eliminated the significant association between PTB and maternal hypertension detected in a previous study [132]. Because obesity is a risk factor for preeclampsia and accounts for about 20% of preeclampsia [142], BMI at age 25 was used as a proxy for pre-pregnancy BMI in the current analysis and results did not change suggesting that our findings may be independent of this potential confounder. In the race/ethnicity-specific analysis, the ORs of hypertension were similar across white, black, and Hispanic groups for women with a prior preterm-LBW delivery (ORs ranging from 1.22 to 1.49). This study detected borderline statistically significant increased odds of hypertension in white (adjusted OR, 1.39; 95% CI, 0.92-2.10) and black women (adjusted OR, 1.49; 95% CI, 0.93-2.38), but did not detect any difference in hypertension among Hispanic women. Unlike a previous study that reported a significantly higher risk of developing hypertension after a PTB in Danish women [132], the estimates in the current study did not reach statistical significance in white women. It may be due to the nature of the NHANES data collection. In the present study, PTBs delivered with BW above 2,500 g were categorized in the BW of ≥2,500 g group (reference group), which might dilute the effect. Another possible reason why the current study

did not detect such effects was that this study had a smaller sample size of white women than the previous Danish study [132].

Growth restriction is positively associated with maternal cardiovascular morbidity and mortality [87]. During pregnancy, the maternal cardiovascular system undergoes hemodynamic changes to facilitate placental circulation in order to guarantee fetal oxygen and nutrition supply. Women at risk of CVD may have an impaired ability to adjust to this hemodynamic challenge and be at higher risk of placental dysfunction, the most common cause of intrauterine growth restriction and a common feature of preeclampsia. The race/ethnicity-specific analyses suggested that unlike the similar ORs of hypertension across three race/ethnicity groups found in women with a prior preterm-LBW delivery, white, black, and Hispanic women experienced different risks of hypertension after a SGA-LBW delivery. Black women with a prior SGA-LBW delivery were about twice as likely as women with a prior BW>2,500g delivery to have hypertension (adjusted OR, 2.09; 95% CI, 1.12-3.90). No differences in hypertension were detected for white or Hispanic women after a SGA-LBW delivery. Taken together, these findings suggest that vascular dysfunction may be of particular importance in the linkage between LBW and hypertension among black women.

The associations of LBW with subsequent maternal hypertension especially among pre-menopausal women or those with a shorter duration since last pregnancy suggested that divergent pathways may link LBW and subsequent maternal risk of hypertension among young/pre-menopausal women compared to older women. Over the last two decades, there have been significant changes in the characteristics of women of reproductive age in the United States. There is an increase in women delaying childbirth into their third or fourth decade of life and an increase in the prevalence of pre-pregnancy obesity. Thus, the more recent cohort of pregnant

women may be at higher risk for both LBW and hypertension than the earlier cohorts. This may explain the more pronounced association between LBW and hypertension among the younger women in the current study.

Hypertension has a well-established association with clinical cardiovascular disease. However, measures of resting BP assessed on a continuous scale can also be informative, because even within the normotensive range, increased resting BP is a major independent risk factor for future coronary heart disease. Women who delivered a Preterm-LBW or SGA-LBW infant had significantly higher SBP compared to women with a BW≥2,500 g delivery. Race/ethnicity did not significantly modify the association between LBW delivery and SBP or DBP throughout the range of blood pressure. The cut-off values used in the hypertension definition to categorize hypertension as a dichotomous variable may partly explain why race/ethnicity was a significant effect modifier in the multiplicative model but not in the additive model. Sensitivity analyses conducted among the women who were not taking anti-hypertensive medication at interview showed minimal impact on estimates. Longitudinal studies have documented that even modest decreases in the BP in the general population have the potential to substantially reduce morbidity and mortality or at least delay the onset of hypertension [28]. It has been estimated that a 2-3 mm Hg reduction of SBP in the population would result in a 6-9% overall reduction in mortality due to stroke and a 4-6% reduction in mortality due to coronary heart disease [125]. Thus, the modest differences in SBP detected in the current study among women with LBW deliveries may contribute to excess CVD.

Strengths and Limitations

Findings of the current study must be considered in light of limitations. First, the pregnancy history data was self-reported and collected retrospectively. However, maternal

recalled infant BW and gestational age are accurate and reliable when reported years after delivery. There is evidence that only 1.6% of BW would have been misclassified into low, normal or high BW and 16.5% of gestational age would have been misclassified into preterm, term or post-term based on maternal recall [143]. Second, due to data collection questions in NHANES, infants born preterm and with BW≥2,500 g were likely to be moderately preterm (delivered at 35-36 weeks) but were grouped in the reference group. Previous reports indicate increased risk of hypertension after pregnancy among women who delivered even moderately preterm infants [87], so this misclassification might bias the associations towards the null. Meanwhile, SGA infants who were born preterm were grouped into the Preterm-LBW group and these mothers may be more severely affected. In the study population, the number of participants in Preterm-LBW (n=565) was about twice the number of participants in the SGA-LBW (n=287), consistent with the expectation that two-thirds of LBW infants would be born preterm [126]. Therefore, the misclassification may not be excessive. Lastly, the cross-sectional nature of NHANES did not allow causal inference in the study. Because NHANES did not collect information before or during pregnancy, residual confounding might remain. BMI at age 25 was used as a proxy for pre-pregnancy BMI in the sensitivity analysis to address the limitation.

The current study had major strengths. The population-based NHANES data facilitated the examination of race/ethnicity-specific maternal hypertension after LBW delivery in a United States representative population. Additionally, the combination of 8-years of NHANES data provided a large sample size and the standardized examination in NHANES secures high precision of the outcome measurements. To overcome the potential underestimates of hypertension by using self-report alone [144], the outcome measurements in this study included both clinical examination and self-report data. Moreover, this study controlled for several

potential confounding variables (such as cigarette smoking, waist circumference and diet characteristics), which were not available in many previous large registry-based studies.

Conclusions

In summary, this study demonstrated that odds of hypertension were increased in women with a history of LBW delivery in a representative United States population. This association was particularly important for black women. Preterm or SGA delivery may identify women who could benefit from hypertension assessment and CVD prevention to reduce future morbidity and mortality, and this early marker may be of particular importance for black women.

3.0 MATERNAL SERUM INTERLEUKIN-6 AND C-REACTIVE PROTEIN CONCENTRATIONS IN WOMEN WITH A PREVIOUS PRETERM DELIVERY

ABSTRACT

The purpose of this study is to examine associations between preterm birth and maternal interleukin-6 (IL-6) and C-reactive protein (CRP) concentrations eight years after delivery. This study included 316 women (spontaneous preterm birth, n=91; medically indicated preterm birth, n=19, and term birth, n=206) who gave birth between 1997 and 2002, were enrolled in the Women and Infant Study of Healthy Hearts (WISH) study, and had inflammatory markers (IL-6 and CRP) measured between 2006 and 2009. Linear regression models were used to assess if a previous preterm-birth was associated with higher IL-6 or CRP concentrations. We found increased IL-6 concentrations among mothers with a previous spontaneous preterm birth compared to term delivery (2.18 pg/ml vs. 1.82 pg/ml, $p<0.05$), after adjusting for age at study visit, race, education, additional preterm-births, oral contraceptive use, medication use for chronic diseases, menopause status, cigarette smoking history, years between the index pregnancy and study visit, pre-pregnancy body mass index (BMI), weight gain since pre-pregnancy, and BMI at study visit. Moreover, we found that the estimates of association between spontaneous preterm birth and IL-6 concentrations were underestimated before adjusting for adiposity evaluated before and after pregnancy. We did not find significant differences in CRP concentrations among women with previous preterm and term deliveries. The findings highlight

inflammation as a potential pathway linking spontaneous preterm delivery and maternal cardiovascular risk later in life.

3.1 INTRODUCTION

Studies have identified increased risk of coronary heart disease, hypertension, and metabolic syndrome in women with a previous preterm delivery [81,87,131,132,145]. While these epidemiological findings are strong and consistent, an understanding of the underlying mechanisms is currently lacking. A key role of low-grade inflammation has been postulated in the development and progression of atherosclerotic disease [104]. Chronically higher concentrations of C-reactive protein (CRP), a sensitive marker of inflammation regardless of etiology [146], add additional information to predict future cardiovascular events, after initial screening with conventional risk factors alone [106,107,147]. The Reynolds Risk Score incorporating CRP has been proposed for cardiovascular risk assessment in women [111]. IL-6 is produced by a variety of cells in response to infection or during chronic inflammation. Adipose tissue is a major determinant of plasma IL-6 concentrations, contributing as much as 30% of total body production [148,149]. IL-6 is a primary determinant of hepatic production of CRP [150-152]. In addition, IL-6 has also been proposed to directly and indirectly affect glucose homeostasis and metabolism [153]. Therefore, plasma IL-6 may have an important role distinct from CRP in the pathogenesis of atherosclerosis and cardiovascular diseases. IL-6 concentrations are moderately correlated with CRP concentrations in healthy adults and in patients with various conditions, with correlation coefficients typically near 0.5 [154-157].

In parallel to work linking inflammation to cardiovascular disease, studies have shown that serum CRP [96-98] and IL-6 [31] during pregnancy are linked to spontaneous preterm delivery (sPTB), which accounts for about 70% of all singleton preterm births (PTB) [31]. The other 30% are medically indicated preterm births (mi-PTB) with preeclampsia as one of the leading causes [11,99]. Preeclampsia is also associated with higher CRP and IL-6 concentrations during [100,101] and after [102] pregnancy. The association between a previous PTB and CVD may be, in part, mediated through chronic activation of inflammatory pathways [128]. However, the relationship of previous preterm delivery and subsequent circulating inflammatory marker concentrations is unknown. One retrospective cohort study suggested that women who experienced PTB, specifically mi-PTB, had increased CRP concentrations in later life compared to women who delivered at term [103]. In recognition that IL-6 is a major determinant of CRP production in liver [158] and that IL-6 may be a better marker of subclinical and clinical cardiovascular diseases than CRP [159-162], it is not known yet whether the increased concentration of CRP in women with PTB is related to IL-6.

Adiposity is strongly related to inflammation and has a key role in PTB. Pre-pregnancy body mass index (BMI) is negatively associated with weight gain during pregnancy[163] but positively associated with weight retention after pregnancy [164]. Women with lower pre-pregnancy BMI have increased risk of spontaneous PTB [165]. Therefore, we propose that pre-pregnancy BMI may have negative confounding effects on the association between a prior sPTB and subsequent maternal inflammatory status, perhaps masking a true association (Figure 4).

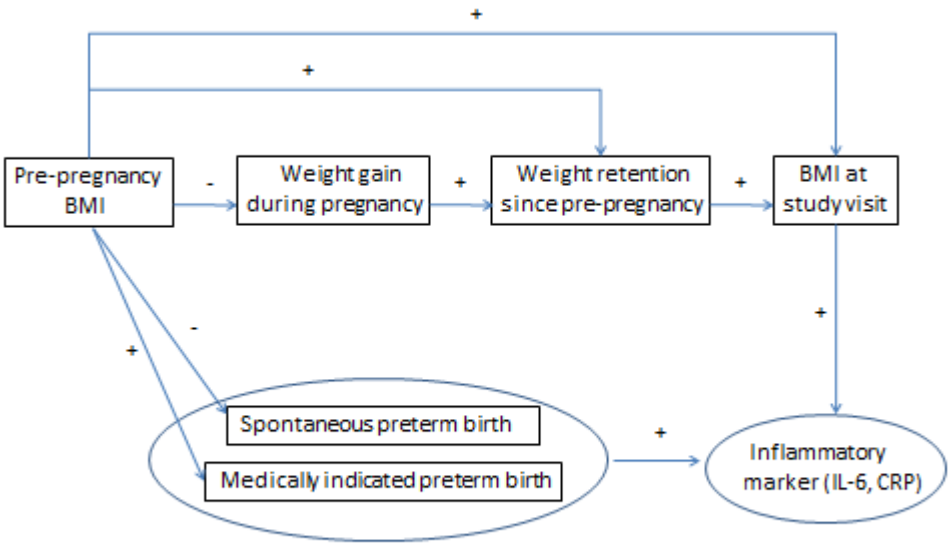

Figure 4. Directed acyclic graph of the potential negative confounding role of pre-pregnancy BMI and weight retention after pregnancy in the association between preterm birth and inflammatory markers.

We examined circulating IL-6 and CRP concentrations in relation to a previous PTB and hypothesized that concentrations of both inflammatory markers will be increased after a previous PTB delivery. We studied spontaneous PTB and medically indicated PTB separately as the etiology of each is thought to be distinct. Given the key role of adiposity in PTB and low-grade chronic inflammation [166], the second aim of the study was to examine the impact of pregnancy-related weight factors on the above associations. We hypothesized that pre-pregnancy body mass index may have negative confounding effects on the association between a previous PTB and subsequent maternal inflammatory status, resulting in underestimates before adjustment of pregnancy-related weight characteristics.

3.2 METHODS

3.2.1 Study population

The Women and Infant Study of Healthy Hearts (WISH) study is a cohort study of cardiovascular risk factors assessed among women after delivery of singleton infants. The University of Pittsburgh Institutional Review Board approved all study procedures. Eligible women were those who gave birth to an infant between 1997 and 2002 at Magee-Womens Hospital in Pittsburgh, Pennsylvania, following a pregnancy not complicated by preeclampsia, pre-pregnancy hypertension, or diabetes. Women were enrolled between 2006 and 2009 (4-12 years after the index pregnancy), and by design those with preterm (<37 weeks of gestation) or small-for-gestational-age (SGA, $<10^{th}$ percentile based on Magee-Womens hospital reference) births were oversampled. Of the 4,908 women identified as eligible via a hospital electronic birth

registry, 1,569 (32%) were screened by mail or phone. Of the women screened, 702 (45%) provided informed consent and were enrolled. In the current study, those who delivered SGA infants at term (n=192) were excluded from the analysis, as the pathways involved in term SGA and PTB are distinct, and therefore the post-pregnancy inflammation status may also be different. We also excluded women who reported their race or ethnicity as other than white or African American (n=12), women with gestational diabetes (n=11), women whose serum samples were not available or insufficient for inflammatory marker assessment (n=83), and those whose pre-pregnancy weights were not available (n=88). The analytic data set included 316 women.

3.2.2 Study variables

Delivery characteristics

Delivery characteristics were abstracted from hospital birth records. Gestational age was determined mainly by prenatal ultrasound, and preterm births were those delivered before 37 completed weeks. Women were categorized as having delivered spontaneous preterm (following spontaneous premature membrane rupture or preterm labor, n=91), medically indicated preterm (due to placenta previa or abruption, suspected growth restriction, and other fetal or maternal conditions, n=19), or term birth (n=206). The time interval between the subjects' index delivery and WISH visit was 8 years on average (standard deviation=1.8 year).

IL-6 and CRP measurements

Fasting blood samples were collected for IL-6 and CRP measurements. IL-6 (pg/ml) was measured at Magee-Womens Research Institute in Pittsburgh, Pennsylvania using Quantikine HS

ELISA kit from R&D Systems, Inc. (Minneapolis, MN). CRP (mg/l) measurements were completed at the University of Pittsburgh's Nutrition Lab in the Department of Epidemiology. CRP was measured using reagents obtained from Olympus America, Inc. (Melville, NY).

Demographic, lifestyle, and pregnancy characteristics

Women self-reported age at delivery, race (white, African American), education (less than high school, high school, college, more than college), income (less than $20,000, $20,000-$50,000, $50,000 to $100,000, and more than $100,000), insurance (Medicaid, Medicare, private), parity (primiparous, multiparous), oral contraceptive use (yes, no), and menopause status (no menstrual period in the last 12 months or hysterectomy) at the WISH study visit. Physical activity was assessed with the Pfaffenberger Physical Activity Scale and was reported as MET hours/week. Cigarette smoking was assessed during pregnancy and at the WISH study visit, and was categorized to three groups: current smoker, smoker during pregnancy but not current smoker, and never. At the WISH visit, women also self-reported medications that they were taking for heart disease, blood pressure, thyroid disease, cholesterol, and other conditions.

Weight related variables

In the current study, we collected three pregnancy-related weight variables: pre-pregnancy weight, weight change during pregnancy, and weight gain since pregnancy. Height, weight, and waist circumference were measured at the WISH visit. Pre-pregnancy weight was abstracted from medical records (when available, n=292) or self-report (when not available in the medical record, n=24). BMI was calculated as weight (in kilograms) divided by height (in meters) squared (kg/m^2). The participants were divided into three groups according to the World Health Organization BMI categories: normal weight ($\leq$25 kg/m^2), overweight (25 to 30 kg/m^2),

and obese (>30 kg/m^2). Due to small numbers, underweight women (pre-pregnancy BMI<18.5, n=12) were combined with the normal weight category.

Weight gain during the index pregnancy was abstracted from medical records (when available, n=285) or was recalled by participants at the study visit (n=31) [167]. We defined the adequacy of gestational weight gain as inadequate, adequate, or excessive that accounted for gestational age using a method described in detail by Bodnar et al [168]. Weight change since pregnancy was calculated as percent change in weight from pre-pregnancy to study entry (100 * (weight at study entry – pre-pregnancy weight)/pre-pregnancy weight) and was a continuous variable.

3.2.3 Statistical analysis

We used chi-square test to determine differences in categorical maternal characteristics across birth characteristics (spontaneous PTB, medically indicated PTB, and term non-SGA births) and ANOVA to determine differences in continuous maternal characteristics. For characteristics that were significantly different among groups, Tukey's test was conducted for multiple comparisons between sPTB or mi-PTB and term delivery. IL-6 and CRP exhibited skewed distribution, thus we tested for equality in the medians using the Kruskal-Wallis one-factor analysis of variance. Spearman's rank correlation coefficient was used to assess correlation between IL-6 and CRP concentrations and BMI.

The univariate associations between maternal characteristics and IL-6 or CRP (log transformed) were explored with linear regression. Outliers were defined as >3 standard deviation above the mean of log-transformed CRP [169] and IL-6 [170], and no outliers were

detected for IL-6 or CRP. To facilitate interpretation, we converted the coefficient estimates from the linear regression models back into the IL-6 and CRP original scales and these are presented as the geometric means of IL-6 and CRP for each maternal characteristic. Geometric mean is suggested to be a better measure of central tendency for positively skewed data [21].

To test for an independent association between previous PTB delivery and inflammatory marker concentrations, multiple linear regression models were used to determine the difference in log (IL-6) or log (CRP) concentrations for all PTBs, spontaneous PTB, and medically indicated PTB groups, respectively, compared to term births. Maternal characteristics that were associated with birth characteristics, IL-6 or CRP significantly in the univariate analysis were considered as potential confounding variables. Education, income, and insurance were all associated with IL-6 and CRP. We selected education to reflect the participants' socioeconomic status because education information was available for all participants, and education may be the most important socioeconomic status variable associated with both PTB [171] and CVD [172]. Because weight gain during pregnancy, weight gain since pre-pregnancy, and BMI/waist circumference at WISH visit might represent intermediate end points or pathways rather than confounders for our study, we adjusted for these variables in separate models. Linear regression models were constructed as follows: model 1 controlled for all non-weight confounders (age, race, education, additional preterm births, oral contraceptive use, menopause, smoking history, and years since index pregnancy); models 2-5 sequentially controlled for weight variables, including pre-pregnancy BMI, weight gain since pre-pregnancy, Institute of Medicine (IOM) categorization of weight gain during pregnancy, BMI/waist circumference at WISH visit. Akaike information criterion **(**AIC) was used to determine if a model significantly improved compared to the previous model. If one weight variable statistically improved the model, it was retained.

Because BMI and waist circumference at WISH visit were highly correlated (Pearson correlation coefficient=0.92, $p<0.01$) and both represented the participants' adiposity status, we used AIC to assess which variable most improved model fit. In the results, we presented models that were significantly improved from the previous models. To determine if pre-pregnancy BMI and BMI at WISH visit modified the association between the birth characteristics and IL-6 or CRP concentrations, we tested significance of the cross-product terms, at p-value of 0.10. We conducted sensitivity analysis among women who had never smoked before WISH visit.

To test our hypothesis that pre-pregnancy BMI may negatively confound the association between PTB and inflammatory marker concentrations [107,163-165], we tested: 1) if pre-pregnancy BMI were positively associated with inflammatory status; 2) if pre-pregnancy BMI was positively associated with BMI at WISH visit; and 3) if pre-pregnancy BMI was negatively associated with sPTB.

All *P* values presented are 2-tailed; $P<0.05$ was considered statistically significant. All statistical analyses were performed using SAS software, version 9.3 (SAS Institute Inc, Cary, NC).

3.3 RESULTS

Women enrolled in the current study were, on average, 38 years old (ranged from 21 to 55 years) at the study visit. About 75% of the women were white; and 25% were African Americans. Overall, women were normal weight before pregnancy, gained excessive weight during pregnancy, had more than one birth, never smoked cigarettes, and had education at college or above (Table 4). Mothers who delivered mi-PTB tended to have had more preterm-birth

deliveries and had a shorter interval from the index pregnancy to the WISH visit than mothers who delivered term. The medians (interquartile ranges) for IL-6 was 1.40 pg/ml (0.83 pg/ml-2.05 pg/ml) and CRP was 2.04 mg/l (0.80 mg/l -4.21 mg/l). Approximately 35% of the study population had CRP concentrations above 3 mg/l. There were no significant differences in unadjusted median IL-6 or CRP concentrations by the three birth characteristics groups.

Table 4. Maternal characteristics of the study population (n=316).

	sPTB (n=91)	mi-PTB (n=19)	Term (n=206)
Index pregnancy (1997-2002)			
Age (year), *mean ± standard deviation*	29.5±6.6	32.5±6.8	29.0±6.7
African Americans, *n (%)*	28 (31%)	6 (32%)	45 (22%)
Pre-pregnancy BMI, *n (%)*			
<24.9 kg/m^2	65 (71%)	12 (63%)	125 (60%)
25.0-29.9 kg/m^2	17 (19%)	5 (26%)	55 (27%)
≥30.0 kg/m^2	9 (10%)	2 (11%)	26 (13%)
IOM categorization of weight gain during pregnancy, *n (%)*			
Inadequate	17 (19%)	3 (16%)	30 (15%)
Adequate	22 (24%)	3 (16%)	72 (35%)
Excessive	52 (57%)	13 (68%)	104 (50%)
Multiparous	78 (86%)	16 (84%)	183 (89%)
Additional preterm births	12 (13%) †	9 (47%) †	10 (5%) *
Study visit (2005-2009)			
Time since index pregnancy (year)	8.8±1.7	7.6±1.5 †	8.0±1.8 *
Age (year)	38.3±6.8	40.1±7.1	37.1±7.3
Tobacco use history			
Current smoker	29 (32%)	8 (42%)	41 (20%)
Not current smoker but smoker during pregnancy	3 (3%)	0	8 (4%)
Not current smoker/not smoker during pregnancy	59 (65%)	11 (58%)	157 (76%)
BMI, *n (%)*			
<24.9 kg/m^2	43 (47%)	8 (42%)	85 (41%)
25.0-29.9 kg/m^2	27 (30%)	6 (32%)	65 (32%)
≥30.0 kg/m^2	21 (23%)	5 (26%)	56 (27%)
Waist circumference (cm)	90.3±13.8	92.9±16.7	92.0±15.1
Weight change in % since pre-pregnancy	12.4±14.3	10.5±9.6	11.8±14.3

Table 4 (continued).			
	sPTB (n=91)	mi-PTB (n=19)	Term (n=206)
Oral contraceptive use	12 (13%)	1 (5%)	34 (17%)
Medications for chronic diseases	57 (63%)	13 (68%)	118 (57%)
Menopause	8 (8%)	3 (16%)	10 (5%)
Physical activity (MET-h/wk)	12.3±14.4	9.1±9.1	17.7±38.8
Education			
Less than high school	4 (5%)	1 (5%)	8 (4%)
High school	22 (24%)	8 (42%)	55 (27%)
College	51 (56%)	10 (53%)	116 (56%)
More than college	14 (15%)	0 (0)	27 (13%)
Income			
Less than $50,000	42 (50%)	12 (63%)	78 (40%)
$50,000 or more	42 (50%)	7 (37%)	119 (60%)
Insurance			
Medicaid	9 (11%)	1 (6%)	33 (17%)
Medicare	4 (5%)	0	5 (3%)
Private	70 (84%)	16 (94%)	158 (80%)
IL-6 (pg/ml), *median (interquartile range)*	1.52 (0.96-2.50)	1.64 (0.75-2.13)	1.30 (0.79-1.93)
CRP (mg/l), *median (interquartile range)*	1.82 (0.80-4.43)	2.45 (0.79-3.77)	2.07 (0.81-4.33)
Systolic blood pressure (mm Hg)	107.2±12.7	111.8±18.9 †	105.2±9.9 *
Diastolic blood pressure (mm Hg)	70.8±7.9	73.9±12.6	69.8±7.9
Total cholesterol (mg/dL)	198.3±37.2 †	217.4±41.8 †	184.3±39.6 *
HDL cholesterol (mg/dL)	57.9±14.7	63.7±11.7	57.2±13.9
LDL cholesterol (mg/dL)	117.4±31.8	130.9±32.3 †	106.3±32.6 *
ApoB (mg/dL)	88.8±23.3	95.3±27.7	82.9±23.1 *
Triglyceride* (mg/dL) *median (interquartile range)*	98.0 (71.0-141.0)	88.0 (64.0-157.0)	84.0 (64.0-122.0)

Abbreviations: BMI, body mass index; CRP, C-reactive protein; IL-6, interleukin-6; IOM, Institute of Medicine; sPTB, spontaneous preterm birth; mi-PTB, medically indicated preterm birth.
* $p<0.05$ from $\chi 2$ or ANOVA test for overall test.
† $p<0.05$ from $\chi 2$ or Tukey's test for pairwise comparison.
Values are n (%), mean±standard deviation, or median (interquartile range).
Missing data were as follows: 16 were missing for income and 20 were missing for insurance.

African American mothers tended to have higher IL-6 and CRP, as did those with higher pre-pregnancy BMI, more weight gain during pregnancy, higher BMI at WISH visit, higher waist circumference, more weight gain since pre-pregnancy, less education, less income,

cigarette smoking history, and those insured by Medicaid (Table 5). There was a statistically significant, moderate positive correlation between IL-6 and CRP. (Spearman's rank correlation coefficient=0.47, P-value<0.01).

Table 5. Unadjusted comparisons of IL-6 and CRP concentrations by maternal characteristics (n=316).

	n	IL-6, pg/ml	CRP, mg/l
Index pregnancy (1997-2002)			
Age (y)	316	0.99	1.00
Race			
African Americans	79	1.88 *	2.44 *
White	237	1.21	1.72
Pre-pregnancy BMI			
<18.5 kg/m^2	12	0.80 *	0.84 *
18.5-24.9 kg/m^2	190	1.14	1.48
25.0-29.9 kg/m^2	77	1.75	2.48
≥30.0 kg/m^2	37	2.25	4.57
IOM categorization of weight gain during pregnancy			
Inadequate	50	1.09 *	1.32 *
Adequate	97	1.28	1.80
Excessive	169	1.49	2.12
Parity			
Primiparous	39	1.46	1.88
Multiparous	277	1.34	1.88
Additional preterm births			
Yes	31	1.79 *	1.72
No	285	1.31	1.90
Study visit (2005-2009)			
Age (y)	316	0.99	0.99
Cigarette smoke history			
Current smoker	78	1.82 *	2.44 *
Not current smoker but smoker during pregnancy	11	1.57	2.44
Not current smoker/not smoker during pregnancy	227	1.21	1.68
BMI			
18.5-24.9 kg/m^2	136	0.94 *	1.07 *
25.0-29.9 kg/m^2	98	1.55	2.29
≥30.0 kg/m^2	82	2.05	3.71
Waist circumference (cm)	316	1.02 *	1.04 *

Table 5 (continued).			
	n	IL-6, pg/ml	CRP, mg/l
Weight change in % since pre-pregnancy	316	1.02 *	1.03 *
Oral contraceptive use			
Yes	47	1.01 *	3.71 *
No	269	1.42	1.67
Medications for chronic diseases			
Yes	188	1.34	2.34 *
No	128	1.38	1.35
Menopause			
Yes	21	1.63	1.86
No	295	1.34	1.88
Physical activity (MET-h/wk)	316	1.00	1.00
Education			
Less than high school	13	2.66 *	2.89
High school	85	1.57	2.14
College	177	1.31	1.80
More than college	41	0.90	1.48
Income			
Less than $50,000	132	1.77 *	2.41 *
$50,000 or more	168	1.09	1.52
Insurance			
Medicaid	43	1.99 *	2.39
Medicare	9	1.72	1.82
Private	244	1.25	1.79

For categorical maternal characteristics, geometric means of IL-6 or CRP were calculated by back-transforming the mean of log-transformed values. For example, geometric mean of IL-6 in African American was 1.88 pg/ml.

For continuous maternal characteristics, ratios of geometric means of IL-6 or CRP for one-unit increase were calculated by back-transforming the mean of log values. For example, at one-unit increase in age at index pregnancy, a 1% decrease in IL-6 is expected.

*P<0.05 for comparison test within each maternal characteristic.

Missing data were as follows: 16 were missing for income and 20 were missing for insurance.

In the unadjusted analysis, there was no difference in IL-6 concentrations between women who delivered a preterm infant and women who delivered a term infant. Results were not meaningfully different after adjusting for age, race, education, additional preterm-births, menopause status, oral contraceptive use, medication use for chronic disease, cigarette smoke

history, and years between the index pregnancy and WISH visit (Table 6, model 1). The AICs suggested that adding pre-pregnancy BMI, weight gain percentage since pre-pregnancy, and BMI at WISH visit improved the model significantly, and each was added sequentially. Adjustment for pre-pregnancy BMI strengthened the association between sPTB and high IL-6 (sPTB vs. term: 2.36 pg/ml vs. 1.90 pg/ml, $p<0.05$, Table 6, model 2). This association remained after additional adjustment for weight gain since pre-pregnancy and BMI at WISH visit (sPTB vs. term: 2.20 pg/ml vs. 1.82 pg/ml, $p<0.05$, Table 6, model 4). There was no evidence of effect modification by pre-pregnancy BMI or BMI at WISH visit. We did not detect associations between mi-PTB and IL-6 concentrations. In contrast to the IL-6 findings, neither women with sPTB nor women with mi-PTB had increased CRP concentrations 8 years after delivery (Table 7). Sensitivity analyses limited to women who had never smoked showed minimal impact on the estimates.

Table 6. Associations between IL-6 concentrations and birth characteristics (n=316).

	Unadjusted	Model 1	Model 2	Model 3	Model 4
	Geometric mean	Geometric mean	Geometric mean	Geometric mean	Geometric mean
All PTB	1.51	1.70	2.12	2.08	2.01
sPTB	1.55	1.86	**2.29 †**	**2.27 †**	**2.18 †**
mi-PTB	1.36	1.30	1.57	1.57	1.51
Term	1.27	1.58	1.93	1.90	1.82

Abbreviations: PTB, preterm birth; sPTB, spontaneous preterm birth; BMI, body mass index.
†$p<0.05$ compared to term group.
Model 1: adjusted for age at WISH visit, race, education, additional preterm-births, menopause status, oral contraceptive use, cigarette smoking history, medication use for chronic diseases, and years between the index pregnancy and WISH visit.
Model 2: adjusted for pre-pregnancy BMI, in addition to the confounding factors controlled in Model 1.
Model 3: adjusted for weight gain percentage since pre-pregnancy, in addition to the confounding factors controlled in Model 2.
Model 4: adjusted for BMI at WISH visit, in addition to the confounding factors controlled in Model 3.

Table 7. Associations between CRP concentrations and birth characteristics (n=316).

	Unadjusted	Model 1	Model 2	Model 3	Model 4
	Geometric mean	Geometric mean	Geometric mean	Geometric mean	Geometric mean
All PTB	1.86	2.83	4.22	4.14	2.92
sPTB	1.84	3.00	4.26	4.14	2.97
mi-PTB	1.92	2.89	4.01	4.06	2.75
Term	1.88	3.25	4.26	4.10	2.92

Abbreviations: PTB, preterm birth; sPTB, spontaneous preterm birth; BMI, body mass index.
†p<0.05 compared to term group.
Model 1: adjusted for age at WISH visit, race, education, additional preterm-births, menopause status, oral contraceptive use, cigarette smoking history, medication use for chronic diseases, and years between the index pregnancy and WISH visit.
Model 2: adjusted for pre-pregnancy BMI, in addition to the confounding factors controlled in Model 1.
Model 3: adjusted for weight gain percentage since pre-pregnancy, in addition to the confounding factors controlled in Model 2.
Model 4: adjusted for waist circumference at WISH visit, in addition to the confounding factors controlled in Model 3.

To better understand the impact of weight before, during, and after pregnancy on the association between sPTB and inflammatory marker concentrations, we then further explored the possibility that pre-pregnancy BMI may negatively confound the association between sPTB and inflammatory marker concentrations, as adjustment for pre-pregnancy BMI made the associations between sPTB and IL-6 concentrations move further away from the null. In the current study population, pre-pregnancy BMI was positively associated with IL-6 and CRP (Spearman's rank correlation coefficient=0.41, p-value<0.01; 0.41, p-value<0.01, respectively); pre-pregnancy BMI was positively associated with BMI at WISH visit (Pearson correlation coefficient=0.83, p-value<0.01); and pre-pregnancy BMI was negatively associated with sPTB (OR: 0.60, 95% CI: 0.32-1.11, p-value=0.10). Weight retention since pre-pregnancy also contributed to the association between sPTB and IL-6. However, weight gain during pregnancy did not meaningfully impact these associations.

3.4 DISCUSSION

The major finding of our study was that IL-6 concentrations were significantly higher in women eight years after a previous spontaneous preterm vs. term birth after adjustment for demographic characteristics, pre-pregnancy BMI, weight gain since pre-pregnancy, and BMI at WISH study visit. We found no significant difference in CRP concentrations after a previous preterm birth. Our results suggest that inflammation may be a pathway linking preterm birth to later maternal cardiovascular diseases or metabolic syndrome. Moreover, we found that the association between sPTB and IL-6 concentrations was underestimated before adjustments for adiposity evaluated before and after pregnancy.

Consistent with two previous studies [124,173], we did not detect higher CRP concentrations after PTB. One previous study of 1,124 women (30 of whom delivered sPTB; 22 of whom delivered mi-PTB; and 1,072 of whom delivered at term) from Scottish Health Survey reported increased CRP concentrations in women who had delivered mi-PTB infants.[103] The finding that CRP was not associated with previous preterm birth in the current study might be a consequence of limited statistical power (we had similar number of mi-PTB and smaller number of term births than the Scottish study), different definitions of mi-PTB (by design, women with preeclampsia or gestational diabetes were not enrolled in the current study), shorter follow up time (8 years vs. 13 years), and different demographic characteristics of the study populations (such as age and race/ethnicity mix). Ideally, replication of these associations in larger cohorts with detailed repeated measurements of weight and CRP would be useful.

Although epidemiologic evidence has linked PTB with excess maternal CVD risk, few studies have focused on the potential mechanisms linking these conditions. In the cardiovascular context, IL-6 is an important activator of immune cells, may be important in the destabilization

of atherosclerotic plaque, and has been linked to endothelial dysfunction [174-177]. Our current findings of increased IL-6 concentrations after sPTB suggest that systemic low grade inflammation may link some adverse pregnancy outcomes and later CVD.[102,103] Our current study cannot evaluate whether higher IL-6 concentrations preceded or were altered by sPTB, but we propose that pre-pregnancy pro-inflammatory status may be related to sPTB during reproductive years, persist postpartum, and be related to increased CVD risk later in life. Higher IL-6 concentrations in women with a prior PTB may reflect underlying cardiometabolic comorbidities or a cumulative effect of adverse health events. This is supported by previous studies that reported higher blood pressure and high intima-media thickness (IMT) in women following a preterm-birth or preeclampsia [132,178]. Chronic inflammation has also been implicated in the pathogenesis of obesity-related insulin resistance [179,180]. Circulating IL-6 concentrations are positively associated with impaired glucose tolerance and insulin resistance [181]. It is possible that IL-6 synthesized after a sPTB exerts metabolic effects, in addition to promoting immunological and inflammatory responses. This is consistent with the previous report of dyslipidemia in women with a history of sPTB and mi-PTB in the WISH Study [131]. Clarification of the mechanism responsible for IL-6 production, especially after spontaneous PTB, is particularly important in the light of the role of inflammation in the pathophysiology of future cardiovascular and metabolic diseases.

The current study is the first study to our knowledge to report the pregnancy-related adiposity factors' potential negative confounding effect on the PTB-inflammation association. IL-6 belongs to the family of adipokines, which are cytokines derived from adipose tissue. It is estimated that approximately 30% of circulating IL-6 is secreted by adipose tissue under basal, non-inflammatory conditions [148]. The associations between sPTB and IL-6 concentrations

were underestimated without considering pre-pregnancy BMI and weight gain since pre-pregnancy. The present study supports that previous preterm birth, adipose tissue, and pro-inflammatory cytokines are part of an interrelated network that might eventually lead to cardiovascular disease. Future studies designed to elucidate the associations between PTB and subsequent inflammatory status should collect the information on those pregnancy-related adiposity factors.

IL-6 and CRP concentrations are associated with correlation coefficients typically around 0.5, which is consistent with our current study [159,182]. IL-6 and CRP are correlated with BMI (IL6: Spearman's rank correlation coefficient=0.52, p-value<0.01; CRP: 0.53, p-value<0.01) and waist circumference (IL6: Spearman's rank correlation coefficient=0.48, p-value<0.01; CRP: 0.53, p-value<0.01) similarly. IL-6, but not CRP, was higher in women with a sPTB vs. term births in the current study. One possible reason for only detecting increased IL-6 concentrations is that IL-6 and CRP may involve different pathologic cardiovascular processes. Cesari et al. found IL-6 to be a better predictor of incident coronary disease than CRP [160]. IL-6, but not CRP, was associated with subclinical cardiovascular disease based on a combination of ankle-brachial blood pressure, carotid artery stenosis and internal and common carotid artery wall thicknesses, echocardiographic abnormalities, and a positive response to the Rose questionnaire for angina pectoris [159,161]. Lee et al.'s study showed that IL-6 was significantly associated with carotid IMT, regardless of CRP [162]. Thus, increased IL-6 concentrations detected in the current study may be an indicator of the early subclinical pathologic cardiovascular processes. This is supported by a previous study conducted with the WISH study population that found higher IMT among women with PTB<34 weeks [178]. The previous Scottish study followed up the women for 13 years to find increased CRP concentrations in women with a mi-PTB [103].

Follow-up studies that evaluate progression of inflammatory marker concentrations according to PTB clinical presentations are needed to better understand their role in the pathophysiology of CVD.

Limitations and strengths

Some methodological considerations warrant mention. Firstly, the inflammatory outcomes we tested could have been influenced by factors that were not measured or measured imprecisely, even though medications that may impact inflammation, such as for heart disease, hypertension, and thyroid diseases were taken into account. We also considered outliers of CRP and IL-6 because they may indicate recent or current acute inflammatory states, such as those associated with common cold. In addition, biomarker concentrations were only measured once and intra-individual variation cannot be accounted for. Assay variability would be expected to bias findings towards the null so the observed associations are potentially underestimations. Secondly, weight change since pre-pregnancy was used to represent weight retention since pre-pregnancy. This variable reflects a combination of retention of gestational weight gain and weight change caused by the lifestyle alternations associated with child-rearing. In addition, the current study excluded preeclampsia by design. Future studies of mi-PTB should be carried out. Furthermore, our findings must be interpreted in the context of modest study power to fully define the nature of the relationship between a prior preterm-birth and biomarker distributions or to detect interactions between biomarkers and clinical covariates.

Characterization of the study population, pregnancy data abstracted from hospital birth records, and the availability of pre-pregnancy BMI, weight gain during pregnancy, and weight change since pre-pregnancy are the main strengths of the study.

Conclusions

Women with a prior spontaneous preterm birth had higher serum IL-6 concentrations 8 years after pregnancy. Longitudinal measurements of IL-6 and CRP during and after pregnancy may help elucidate pathways linking inflammation to adverse pregnancy outcomes and may identify subsets of women that could benefit from early interventions to improve their cardiovascular health.

4.0 VISCERAL ADIPOSITY TISSUE AND CARDIOMETABOLIC AND INFLAMMATORY FACTORS IN WOMEN WITH A PREVIOUS PRETERM DELIVERY

ABSTRACT

This study is an exploratory study within a prospective cohort study to examine the relations between a prior preterm birth and adiposity measures eight years postpartum. This study also aims to investigate how adiposity measures may contribute to the association between a prior preterm birth and subsequent cardiometabolic and inflammatory risk factors.

This study included 89 women (preterm birth, n=40 and term birth, n=49) who gave birth between 1997 and 2002 and had adiposity measures (BMI, waist circumference, visceral adipose tissue, and subcutaneous adipose tissue), cardiometabolic (systolic blood pressure, diastolic blood pressure, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, Apolipoprotein B, and fasting glucose) and inflammatory markers (interleukin-6 and C-reactive protein) measured between 2006 and 2009. We developed linear regression models to estimate the differences in adiposity measures for women who had a PTB vs. term delivery and assess the associations between PTB and maternal cardiometabolic and inflammatory risk factors. We used bootstrapping mediation analysis to investigate if adiposity measures mediate the associations between PTB and cardiometabolic and inflammatory markers.

Our study suggests that visceral adipose tissue (VAT) was higher in women with a prior PTB vs. term delivery among non-obese women after adjusting for body mass index (14.1±7.5 cm^2, p=0.07). VAT may be a potential mediator of the association between PTB and elevated triglycerides later in life (95% confidence interval for the indirect effect of PTB on triglyceride through VAT: 0.0044, 0.1413). In addition, our results suggest that IL-6 may mark an inflammatory pathway linking PTB to cardiometabolic risk independent of adiposity. The current study provides insight on possible pathways linking a prior PTB to maternal cardiometabolic risk after pregnancy. It highlights the importance of monitoring abdominal adiposity in women after a PTB. Future work is warranted to explore underlying explanations for these results.

4.1 INTRODUCTION

Preterm birth (PTB), defined as gestation length less than 37 weeks, occurs in approximately 11% of births in the United States [7]. Studies have identified increased risk of cardiovascular diseases in women with previous preterm births [81,87,131,132,145]. Yet, the underlying mechanisms remain unknown. Studies have demonstrated that PTB is associated with subsequent subclinical cardiometabolic and inflammatory markers of CVD. In a prospective study of non-preeclamptic women, Catov et al. found that PTB predicted higher systolic blood pressure, atherogenic lipids, and carotid intima-media thickness eight years postpartum [131,178]. One retrospective cohort study suggested that women who experienced PTB, specifically medically indicated PTB, had increased C-reactive protein concentrations compared to women who delivered at term [103]. Obesity, usually defined by an excess of body fat, is a major modifiable risk factor for CVD [69]. The most commonly used anthropometric measurement is body mass

index (BMI) [183]. To our knowledge, two studies have investigated if a prior PTB is associated with subsequent obesity [124,184]. Perng et al. reported that PTB did not predict BMI, weight change, or waist circumference by 3 or 7 years postpartum [184]. Another study also suggested no evidence of associations of PTB with BMI or waist circumference 18 years after pregnancy [124]. It is increasingly recognized that body fat distribution is more important than overall excess adiposity in driving CVD risk [69]. Abdominal obesity, in particular visceral fat, is also implicated in metabolic abnormalities that increase CVD risk [70,71]. Excess visceral adiposity could, on the one hand, play a role in the development of an atherogenic metabolic profile but, on the other hand, represent a reliable marker of more primary abnormalities affecting energy partitioning and cardiometabolic risk [69]. In addition, there are race differences in visceral adiposity tissue accumulation, with whites being more prone to visceral adipose tissue deposition than blacks for any level of total body fat [185]. It is unknown if visceral adiposity may contribute to the relationship between a prior PTB and later life cardiometabolic and inflammatory factors.

We conducted an exploratory study within a prospective cohort study to examine the relationship between a prior PTB and adiposity measures (BMI, waist circumference, visceral adiposity tissue, and subcutaneous adipose tissue) about eight years postpartum. The specific goals were 1) to examine if a previous PTB and abdominal adiposity were associated at eight years postpartum. Given the racial differences in visceral adiposity [186], we investigated the race-specific and obesity-specific associations between PTB and visceral adiposity; 2) to study how adiposity measures may contribute to the association between a prior PTB and subsequent CVD cardiometabolic and inflammatory risk factors. We hypothesized that independent of total

body fat, visceral adiposity tissue might contribute to the CVD risk factors in women with a prior PTB.

4.2 METHODS

4.2.1 Study population

Participants in this study were a subgroup from the Women and Infant Study of Healthy Hearts (WISH Study). The WISH Study is a cohort study of cardiovascular risk factors assessed among women on average 8 years after delivery of singleton infants. The University of Pittsburgh Institutional Review Board approved all study procedures. The details of eligibility and enrollment have been described elsewhere [131,178,187]. Of the 4,908 women identified as eligible via a hospital electronic birth registry, 1,569 (32%) were screened by mail or phone. Of the women screened, 702 (45%) provided informed consent and were enrolled. The current study includes women in whom computed tomography (CT) measurements of abdominal adiposity were also collected. A total of 89 women were enrolled for the current study.

4.2.2 Study variables

Demographic and delivery characteristics

Women self-reported their age and race (non-Hispanic white, non-Hispanic African American) at the WISH study visit. Delivery characteristics were abstracted from hospital birth

records. Gestational age was determined mainly by prenatal ultrasound. Preterm births were those delivered before 37 completed weeks. The time interval between the subjects' index delivery and WISH visit ranged from 4 to 12 years.

Anthropometric and abdominal adipose tissue measurements

Height, weight, and waist circumference were measured by a trained study nurse at the WISH study visit. BMI was calculated as weight (in kilograms) divided by height (in meters) squared (kg/m^2). Abdominal visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) areas (in centimeters squared) were measured using CT.[188,189] A cross-sectional area of abdominal fat was assessed with a single CT scan centered upon the L4-L5. Area of adipose tissue was measured electronically by selecting regions of interest defined by attenuation values of -30 to -190 Hounsfield units.

Cardiometablic and inflammatory marker measurements

Fasting blood samples were collected at WISH study visit. All lipid measurements were completed at the Nutrition Laboratory in the Department of Epidemiology at the University of Pittsburgh. Total cholesterol, high-density lipoprotein cholesterol (HDL cholesterol), and triglycerides were measured using standard enzymatic procedures [190-192]. Low-density lipoprotein cholesterol (LDL cholesterol) was estimated using the Friedewald calculation when triglycerides were <400 mg/dl or directly measured when above this cutpoint [193]. Apolipoprotein B (ApoB) was analyzed by using a variation of the Boehringer Mannheim turbidimetric procedure. Glucose was determined by an enzymatic determination [194]. Blood pressure was evaluated as the mean of three measurements following a 10-minute rest. Interleukin-6 (IL-6, pg/ml) was measured at Magee-Womens Research Institute in Pittsburgh, Pennsylvania using Quantikine HS ELISA kit from R&D Systems, Inc. (Minneapolis, MN). C-

reactive protein (CRP, mg/l) measurements were completed at the University of Pittsburgh's Nutrition Lab using reagents obtained from Olympus America, Inc. (Melville, NY).

4.2.3 Statistical analysis

Characteristics of women with a prior PTB or term births were compared by using chi-square and t-test. The distribution of triglycerides, IL-6, and CRP were skewed and therefore were natural log-transformed for analysis. We next estimated the differences in adiposity measures (BMI, waist circumference, VAT, and SAT) for women who had a PTB vs. term delivery. We then stratified the study population by obesity status (BMI>30) and race (non-Hispanic white and non-Hispanic African American) to examine the strata-specific associations. For the strata that we found a significant association between PTB and an adiposity measure, we further explored if and how this adiposity measure may affect the associations between PTB and maternal cardiometabolic and inflammatory risk factors.

Linear regression models were developed to assess the associations between PTB and maternal cardiometabolic and inflammatory risk factors. This study is an exploratory study and due to the modest sample size and similar characteristics between PTB and term birth groups we restricted adjustment to adiposity measures. We conducted a series of models to understand if adiposity measures impacted the association between PTB and CVD risk factors (model 1: unadjusted model, model 2: model 1+VAT, model 3: model 2+BMI, model 4: model2+waist circumference). Potential issues of collinearity were examined by using the tolerance statistic, with ≤ 0.10 indicative of collinearity.

If we found one adiposity measure that eliminated the association between PTB and CVD risk factor, we conducted tests to evaluate if the adiposity may be an effect mediator between PTB and CVD risk factors. Bootstrapping test was used to test mediation [195]. Bootstrapping is an increasingly popular method of indirect effect testing [196]. It is a non-parametric method based on resampling with replacement. From each of these samples the indirect effect is computed and a sampling distribution is generated. A confidence interval is then computed from the distribution. If the confidence interval does not include 0, we could consider the indirect effect is different from 0. In the current study, bootstrapping test tested the significance of indirect effect of a predictor variable (PTB) on the outcome (CVD risk factor) through a hypothesized mediator (adiposity measures).

All *P* values presented are 2-tailed; $P<0.05$ was considered statistically significant and $P<0.10$ was considered borderline statistically significant (to account for the modest sample size). All statistical analyses were performed using SAS software, version 9.3 (SAS Institute Inc, Cary, NC).

4.3 RESULTS

A total of 40 women with PTB and 49 women with term delivery were enrolled for the current study. Women with PTB and term delivery did not differ in terms of age, race, BMI, waist circumference, VAT, SAT, blood pressure, lipids profile, and CRP (Table 8). Women with PTB had higher IL-6 than their term counterparts (median of 1.8 pg/ml vs. 1.1 pg/ml). On average, the women were 40 years old; approximately 75% of the women were non-Hispanic white (white), and 25% were non-Hispanic African Americans (black); waist circumference was 93cm and

BMI was 27.4 kg/m^2. The mean VAT volume was 94.1±53.4 cm^2 and mean SAT volume was 304.4±179.3 cm^2. The study population has similar demographic characteristics as the parent WISH study population except age at enrollment. On average, the women in the current study are younger than the women in the parent WISH study (39.5-year old vs. 37.4-year old).

Table 8. Study population characteristics (n=89).

	PTB (n=40)	Term delivery (n=49)	p-value
Age, years	40.5±6.5	38.7±6.0	0.19
Race			
White	28 (70%)	38 (78%)	0.42
Black	12 (30%)	11 (22%)	
Waist circumference, cm	92.9±14.1	92.4±16.3	0.89
BMI			
Non-obese (BMI<30 kg/m^2)	29 (72%)	35 (71%)	0.91
Obese (BMI>30 kg/m^2)	11 (28%)	14 (29%)	
VAT, cm^2	96.4±51.3	92.3±55.6	0.72
SAT, cm^2	282.3±156.8	322.4±195.5	0.30
SBP, mm Hg	110.3±10.3	106.6±8.8	0.07
DBP, mm Hg	72.5±8.3	71.0±7.7	0.36
Fasting glucose, mg/dL	91.8±19.0	93.4±10.5	0.65
Total cholesterol, mg/dL	197.1±36.3	183.4±39.6	0.10
HDL cholesterol, mg/dL	55.4±13.6	57.0±13.4	0.60
LDL cholesterol, mg/dL	118.2±29.6	104.7±34.4	0.06
Apolipoprotein-B	88.2±23.2	83.4±23.6	0.35
Triglyceride,* mg/dL *median (interquartile range)*	83.0 (58, 130)	92.5 (70.0, 138.0)	0.33
IL-6, * pg/ml *median (interquartile range)*	1.8 (1.1, 2.8)	1.1 (0.5, 2.1)	**0.01**
CRP, * mg/l *median (interquartile range)*	1.2 (0.6, 3.5)	2.2 (0.7, 4.5)	0.36

* The distribution of triglyceride, CRP, and IL-6 was skewed and was presented as median with interquartile range.

Abbreviation: BMI, body mass index; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL cholesterol, high-density lipoprotein cholesterol; LDL cholesterol, low-density lipoprotein cholesterol; IL-6, interleukin-6; CRP, C-reactive protein.

PTB was not related with BMI, waist circumference, VAT, or SAT eight years after delivery (Table 9). In the stratified analysis, non-obese (BMI<30 kg/m^2) women (n=64) had borderline significantly higher VAT after a PTB (14.1±7.5, p=0.07) after adjusting for BMI. We did not detect differences in any adiposity measures in other strata.

Table 9. Associations of PTB with adiposity measures at 8 years postpartum (n=89).

			Stratified by obesity status		Stratified by race	
	Total population (n=89)		Non-obese (n=64)	Obese § (n=25)	White (n=66)	Black (n=23)
	mean±SE (p-value)		mean±SE (p-value)	mean±SE (p-value)	mean±SE (p-value)	mean±SE (p-value)
BMI, kg/m^2	-0.6±1.5 (p=0.71)		0.1±0.8 (p=0.91)	-1.7±2.2 (p=0.43)	0.4±1.6 (p=0.79)	-4.5±3.1 (p=0.16)
Waist circumference, cm *	1.6±1.3 (p=0.22)		1.1±1.4 (p=0.43)	2.0±2.7 (p=0.48)	1.0±1.6 (p=0.51)	4.0±2.4 (p=0.10)
VAT, cm^2 *	7.2±7.7 (p=0.35)		**14.1±7.5 (p=0.07)**	-12.2±19.8 (p=0.51)	8.7±7.1 (p=0.23)	-0.9±19.4 (p=0.96)
SAT, cm^2 *	-27.9±20.2 (p=0.17)		-4.9±15.9 (p=0.76)	-94.8±60.3 (p=0.13)	-19.8±17.4 (p=0.26)	-69.0±62.5 (p=0.28)

Abbreviation: SE, standard error; BMI, body mass index; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue.
*Adjusted for BMI.
§ Obese is defined as BMI>30 kg/m^2.

We then assessed the relationship between PTB and cardiometabolic and inflammatory markers among non-obese women (n=64, Table 10). In the unadjusted analysis, women with a prior PTB had elevated IL-6 concentrations ($p<0.01$) and borderline significantly elevated SBP and triglycerides compared to women with term delivery ($p<0.10$). Additional adjustment for VAT, BMI or waist circumference had minimal impact on the associations between PTB and IL-6 or SBP. However, after adjustment for VAT, the association between PTB and triglycerides was eliminated. We employed the bootstrapping procedure [195] to further understand if VAT mediated the association between PTB and triglycerides. The column data shows the indirect effect estimated from the data in the current study. The column bootstrap, standard error, and 95% confidence interval shows the indirect effect estimated with the bootstrapping methods. Since the 95% confidence interval does not include 0, this test supports a potentially mediating role for VAT between PTB and triglycerides (Table 11 and figure 5. 95% confidence interval for the indirect effect of PTB on triglyceride through VAT: 0.0044, 0.1413).

Table 10. Associations of PTB with cardiometabolic and inflammatory biomarkers in non-obese participants at 8 years postpartum (n=64).

	Model 1, unadjusted model	Model 2, adjusted for VAT	Model 3, adjusted for VAT and BMI	Model 4, adjusted for VAT and waist circumference
	mean±SE (p-value)	mean±SE (p-value)	mean±SE (p-value)	mean±SE (p-value)
SBP, mm Hg	4.3±2.5 (p=0.098)	4.7±2.6 (p=0.07)	4.8±2.6 (p=0.08)	4.6±2.6 (p=0.08)
DBP, mm Hg	1.5±2.0 (p=0.46)	1.4±2.0 (p=0.49)	1.5±2.1 (p=0.48)	1.3±2.0 (p=0.52)
Fasting glucose, mg/dL	1.7±3.0 (p=0.58)	0.7±3.1 (p=0.83)	0.7±3.1 (p=0.83)	0.6±3.1 (p=0.84)
Total cholesterol, mg/dL	13.6±10.1 (p=0.18)	7.6±9.9 (p=0.44)	8.2±9.8 (p=0.41)	7.7±9.9 (p=0.44)
HDL cholesterol, mg/dL	-2.3±3.3 (p=0.50)	-0.4±3.2 (p=0.90)	-0.7±3.2 (p=0.83)	-0.7±3.0 (p=0.82)
LDL cholesterol, mg/dL	11.9±8.5 (p=0.17)	6.6±8.2 (p=0.43)	7.4±8.0 (p=0.36)	6.9±8.2 (p=0.40)
Apolipoprotein-B	3.0±5.9 (p=0.61)	-0.6±5.7 (p=0.91)	-0.05±5.6 (p=0.99)	-0.4±5.7 (0.94)
Triglyceride *	0.22±0.13 (p=0.099)	0.09±0.11 (p=0.41)	0.10±0.11 (p=0.39)	0.09±0.11 (p=0.41)
IL-6 *	**0.56±0.16 (p<0.01)**	**0.52±0.16 p<0.01)**	**0.57±0.16 (p<0.01)**	**0.57±0.16 (p<0.01)**
CRP *	-0.14±0.30 (p=0.65)	-0.26±0.30 (p=0.38)	-0.22±0.28 (p=0.43)	-0.24±0.29 (p=0.41)

Abbreviation: SE, standard error; BMI, body mass index; VAT, visceral adipose tissue; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL cholesterol, high-density lipoprotein cholesterol; LDL cholesterol, low-density lipoprotein cholesterol; IL-6, interleukin-6; CRP, C-reactive protein.
* The distribution of triglyceride, IL-6, and CRP were skewed and log-transformed for analysis.

Table 11. Indirect effects of PTB on triglycerides through VAT in non-obese women (n=64).

	Data	Bootstrap	Standard error	95% confidence interval
VAT	0.0607	0.0559	0.0325	0.0044, 0.1413

Abbreviation: VAT, visceral adipose tissue.

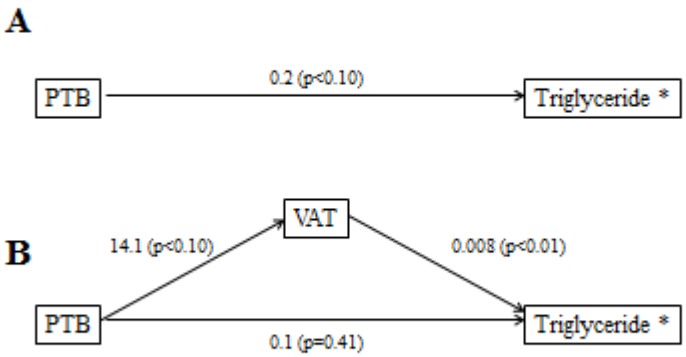

Figure 5. Association between PTB and triglycerides in non-obese women (n=64). Figure 5A depicts the unadjusted model of PTB and triglycerides. Figure 5B depicts the model that includes VAT. *Triglyceride was natural log-transformed for analysis.

In the total study population (APPENDIX, Table 13), in addition to elevated IL-6 levels and borderline significantly higher SBP, we also found that women with a prior PTB had borderline significantly higher LDL-cholesterol after a PTB (13.0±7.1, p=0.07). However, we did not detect any differences in triglycerides. VAT, BMI, and waist circumference made minimal impacts on these associations.

4.4 DISCUSSION

Our study provides insight on possible pathways linking a prior PTB to maternal cardiometabolic risk after pregnancy. Our study suggests that VAT was higher in women with a prior PTB vs. term delivery among non-obese women eight years after delivery. VAT may be a potential mediator on the association between PTB and elevated triglycerides later in life. In addition, our results suggest that IL-6 may mark an inflammatory pathway linking PTB to cardiometabolic risk independent of adiposity.

The other studies of the relations between a prior PTB and BMI, weight change from pre-pregnancy, and waist circumference evaluated postpartum reported that PTB was unrelated to these adiposity measures [124,184]. Similar to these studies, we found no associations of a prior PTB with BMI, waist circumference, or directly quantified abdominal fat depots eight years postpartum. However, when we stratified the population by obesity status, we detected increased VAT after a PTB among non-obese women. Moreover, our study suggested that VAT may mediate the association between a prior PTB and maternal triglycerides level in the non-obese women. We have also explored if VAT might mediate the associations in white, African American women. The current study did not find mediating effects of VAT in the association between PTB and later maternal triglycerides in either of the racial group.

Excess VAT could be considered as a marker of an altered cardiometabolic risk profile predictive of increased CVD risk [189]. If the findings could be validated by future studies, it would be informative to develop new preventive strategies. Studies have confirmed the hypothesis that “hypertriglyceridemic waist”, simultaneous presence of fasting hypertriglyceridemia and an enlarged waist circumference, predicts excess visceral adiposity [197] and is associated with metabolic risk profile changes.[198-200] Therefore, the combination

of waist circumference and triglycerides may provide a convenient tool to screen for the subgroup of women with a prior PTB characterized by excess visceral adiposity. Preventive strategies aimed at reduction of waist circumference and circulating triglycerides levels may improve the post-pregnancy cardiovascular risk trajectory.

Potential mechanisms for the associations we detected are unclear. The question of whether PTB itself modulates the body fat distribution, independently contributes to later higher VAT, or whether the higher VAT observed after PTB exists since pre-pregnancy, is yet to be answered. It would be of great interest to know how increased VAT measured eight years after delivery correlates with overall or abdominal adiposity before and during pregnancy, which could have affected pregnancy outcomes. We considered subtype of PTB (spontaneous PTB or medically indicated PTB) might be an effect modifier for the observed association. The observed effect might be driven by medically indicated PTB. However, due to the small number of medically indicated PTB (n=2) in the current study, we could not further explore this hypothesis. Future studies examining the association between PTB and postpartum VAT in larger cohorts with follow up from pre-pregnancy, during pregnancy, to postpartum are warranted. A better understanding of the biological processes could unveil intervention opportunities and lead to improvements in preventive strategies for both PTB and CVD.

Consistent with previous studies, our current findings of increased IL-6 concentrations after PTB suggest that systemic low grade inflammation may link some adverse pregnancy outcomes and later CVD [102,103]. The present study cannot evaluate whether higher IL-6 concentrations preceded or were altered by PTB, but we propose that pre-pregnancy pro-inflammatory status may be related to PTB during reproductive years, persist postpartum, and be related to increased CVD risk later in life. Clarification of the mechanism responsible for IL-6

production, especially after spontaneous PTB, is particularly important in the light of the role of inflammation in the pathophysiology of future cardiovascular and metabolic diseases.

Our results are exploratory and preliminary. When interpreting the results of this study, it is important to acknowledge the study limitations. First, there is possibility of selection bias because we restricted the analysis to women who participated in the CT measurements of abdominal adiposity. Whether the findings could be replicated in other populations will be essential to examine. Second, the study has modest small sample size. We did not control for some potential confounding variables, such as lifestyle factors, socio-economic factors, and medication use. In future larger studies, potential confounding variables and effect modifiers should be considered. In addition, due to the different etiology of spontaneous PTB and medically indicated PTB, future studies should examine spontaneous PTB separately from medically indicated PTB to gain insight on the mechanisms underlying these pregnancy outcomes and maternal CVD. Finally, the adiposity measures and cardiometabolic factors were measured at the same time. Therefore, the indirect effects that we explored are not strictly mediating effects. The effects need to be validated by future longitudinal studies.

However, our study also has several strengths. To our knowledge, this is the first study to investigate direct measure of visceral adiposity after a PTB, and how VAT could impact the maternal CVD risk profile. The direct measure of visceral adiposity, which has been postulated to be most closely associated with serum lipids, enables us to gain insights into the underlying mechanisms that explain why women with a prior PTB are at higher cardiometabolic risk later in life.

The observed higher VAT eight years after a prior PTB in non-obese women and the potential mediating role of VAT on the association between PTB and triglyceride are new

findings. Abdominal adiposity might contribute to the link between a prior PTB and long term maternal cardiovascular disease risk. The current study highlights the importance of monitoring the abdominal adiposity in women after a PTB. Future work is warranted to explore underlying explanations for these results.

5.0 CONCLUSION

This dissertation aims to provide insights on possible underlying pathways linking a prior LBW delivery to long term maternal cardiovascular health. Our findings suggest that excess CVD risk may be detectable among women with a history of LBW at reproductive age. The potential mechanisms that lead to increased CVD risk from LWB delivery might go through vascular dysfunction, inflammation, adiposity, and lipids.

The major achievements of this dissertation can be summarized in three parts;

(1) *Vascular dysfunction may be one of the mechanisms explaining why women with a prior LBW are at increased CVD risk later life*. This study demonstrated that odds of hypertension were increased in women with a history of LBW delivery in a representative United States population. This association was particularly important in non-Hispanic African American women.

(2) Inflammation may be a pathway linking preterm birth to later maternal cardiovascular diseases or metabolic syndrome. IL-6 concentrations were significantly higher in women eight years after a previous spontaneous preterm vs. term birth after adjustment for demographic characteristics, pre-pregnancy BMI, weight gain since pre-pregnancy, and BMI at WISH study visit. The association between a prior PTB and increased IL-6 concentrations was independent of directly quantified visceral adiposity tissue.

(3) Abdominal adiposity might contribute to the link between a prior PTB and long term maternal cardiovascular disease risk. Our study suggests that visceral adipose tissue was higher in women with a prior PTB vs. term delivery among non-obese women eight years after delivery. Moreover, visceral adipose tissue may be a potential mediator on the association between PTB and elevated triglycerides later in life.

The original contributions of this research can be summarized as follows:

(1) *This is the first study to demonstrate race/ethnicity-specific relationships between a LBW delivery and hypertension after pregnancy.* Evidence from this research suggested that race/ethnicity modified the association between a previous LBW delivery and maternal hypertension, such that risk may be higher among non-Hispanic African American women. This study suggested that while the Preterm-LBW association with maternal hypertension was similar regardless of maternal race/ethnicity, the link with SGA-LBW appeared to be limited to non-Hispanic women.

(2) *The current study is the first study to our knowledge to report the pregnancy-related adiposity factors' potential negative confounding effect on the PTB-inflammation association.* The association between sPTB and IL-6 concentrations was underestimated before adjustments of adiposity evaluated before and after pregnancy.

(3) *Visceral adipose tissue was higher in women with a prior PTB vs. term delivery among non-obese women eight years after delivery*.

(4) *Visceral adipose tissue may be a potential mediator on the association between PTB and elevated triglycerides later in life.*

The findings in this research could be further improved and extended in larger cohorts with detailed measurements of weight and inflammatory markers from pre-pregnancy, during pregnancy, to postpartum.

Public health significance

A better understanding of the biological processes that link LBW to future maternal CVD diseases could unveil intervention strategies for both LBW and CVD. Adverse pregnancy outcomes may be useful for identifying women who could benefit from cardiometabolic risk assessment and primary prevention to reduce future morbidity and mortality. Women with a history of LBW should be encouraged to optimize their lifestyle in order to prevent future CVD and to evaluate and monitor cardiovascular risk factors (hypertension, obesity, inflammation, and dyslipidemia). Possible avenues for CVD prevention include more frequent blood pressure and lipids monitoring and earlier behavioral interventions (e.g., reduction of waist circumference).

APPENDIX. ASSOCIATIONS OF PTB WITH CARDIOMETABOLIC AND INFLAMMATORY BIOMARKERS IN ALL PARTICIPANTS AND IN WHITE WOMEN.

Table 12. Differences in cardiometabolic and inflammatory biomarkers in women with PTB vs. term delivery at 8 years postpartum (n=89).

	Model 1: Unadjusted model	Model 2, adjusted for VAT	Model 3, adjusted for VAT and BMI	Model 4, adjusted for VAT and waist circumference
	mean±SE (p-value)	mean±SE (p-value)	mean±SE (p-value)	mean±SE (p-value)
SBP, mm Hg	3.7±2.0 (p=0.07)	3.6±2.0 (p=0.08)	3.6±2.0 (p=0.08)	3.6±2.0 (p=0.08)
DBP, mm Hg	1.6±1.7 (p=0.36)	1.4±1.6 (p=0.40)	1.5±1.7 (p=0.37)	1.4±1.7 (p=0.40)
Fasting glucose, mg/dL	-1.6±3.3 (p=0.63)	-1.9±3.2 (p=0.54)	-1.9±3.2 (p=0.57)	-1.9±3.2 (p=0.55)
Total cholesterol, mg/dL	13.7±8.3 (p=0.10)	13.3±8.3 (p=0.12)	12.1 ±8.3 (p=0.15)	12.1±8.3 (p=0.15)
HDL cholesterol, mg/dL	-1.6±2.9 (p=0.60)	-0.8±2.6 (p=0.76)	-1.3±2.5 (p=0.61)	-0.9±2.4 (p=0.70)
LDL cholesterol, mg/dL	13.5±7.1 (p=0.06)	13.0 ±7.0 (p=0.07)	12.4±7.1 (p=0.08)	13.0±7.1 (p=0.07)
Apolipoprotein-B	4.8±5.1 (p=0.35)	4.3±5.0 (p=0.40)	4.0±5.1 (p=0.44)	4.3±5.1 (p=0.40)
Triglyceride, mg/dL *	0.1±0.1 (p=0.33)	0.1±0.1 (p=0.41)	0.1±0.1 (p=0.46)	0.1±0.1 (p=0.42)
IL-6, pg/ml *	**0.4±0.2 (p=0.02)**	**0.3±0.2 p=0.02)**	**0.4±0.1 (p<0.01)**	**0.4±0.1 (p<0.01)**
CRP, mg/l *	-0.3±0.3 (p=0.31)	-0.3±0.2 (p=0.18)	-0.2±0.2 (p=0.28)	-0.3±0.2 (p=0.16)

Abbreviation: SE, standard error; BMI, body mass index; VAT, visceral adipose tissue; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL cholesterol, high-density lipoprotein cholesterol; LDL cholesterol, low-density lipoprotein cholesterol; IL-6, interleukin-6; CRP, C-reactive protein.
* The distribution of triglyceride, IL-6, and CRP were skewed and was log-transformed for analysis.

Table 13. Differences in cardiometabolic and inflammatory biomarkers in white women with PTB vs. term delivery at 8 years postpartum (n=66).

	Model 1: Unadjusted model	Model 2, adjusted for VAT	Model 3, adjusted for VAT and BMI	Model 4, adjusted for VAT and waist circumference
	mean±SE (p-value)	mean±SE (p-value)	mean±SE (p-value)	mean±SE (p-value)
SBP, mm Hg	2.8±2.4 (p=0.25)	2.2±2.4 (p=0.35)	2.1±2.4 (p=0.38)	2.1±2.4 (p=0.38)
DBP, mm Hg	2.2±2.0 (p=0.29)	1.5±1.9 (p=0.43)	1.6±1.9 (p=0.42)	1.4±1.9 (p=0.46)
Fasting glucose, mg/dL	1.9±2.9 (p=0.52)	1.3±2.9 (p=0.67)	1.3±3.0 (p=0.66)	1.2±3.0 (p=0.68)
Total cholesterol, mg/dL	17.7±9.5 (p=0.07)	16.9±9.7 (p=0.08)	15.6±9.6 (p=0.11)	16.4±9.6 (p=0.09)
HDL cholesterol, mg/dL	-3.4±3.5 (p=0.35)	-1.5±3.1 (p=0.62)	-2.0±3.1 (p=0.52)	-2.0±2.9 (p=0.50)
LDL cholesterol, mg/dL	15.0±7.8 (p=0.05)	14.1±7.9 (p=0.08)	13.3±7.9 (p=0.10)	13.9±7.9 (p=0.08)
Apolipoprotein-B	7.3±5.6 (p=0.20)	6.0±5.6 (p=0.29)	5.7±5.7(p=0.32)	6.0±5.7 (p=0.29)
Triglyceride, mg/dL *	0.3±0.1 (p=0.06)	0.2±0.1 (p=0.11)	0.2±0.1 (p=0.13)	0.2±0.1 (p=0.11)
IL-6, pg/ml *	**0.5±0.2 (p<0.01)**	**0.4±0.1 (p=0.01)**	**0.4±0.1 (p<0.01)**	**0.4±0.1 (p<0.01)**
CRP, mg/l *	-0.3±0.3 (p=0.31)	-0.4±0.3 (p=0.13)	-0.3±0.2 (p=0.18)	-0.4±0.2 (p=0.14)

Abbreviation: SE, standard error; BMI, body mass index; VAT, visceral adipose tissue; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL cholesterol, high-density lipoprotein cholesterol; LDL cholesterol, low-density lipoprotein cholesterol; IL-6, interleukin-6; CRP, C-reactive protein.
* The distribution of triglyceride, IL-6, and CRP were skewed and was log-transformed for analysis.

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