

Biochemical Effects of Toxic Stress and Its Effect on Inflammation During Pregnancy

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Abstract

Toxic stress is characterized by physical, psychological, and environmental stress that is detrimental to one's health. The stress pathway is believed to be involved in preterm birth via inflammatory and immunologic dysregulation. We conducted a cross-sectional study on female participants aged 15-45 years old who delivered a live, singleton infant at Magee Women's Hospital in Pittsburgh, PA. Each participant was assessed for risk of toxic stress using a combination of individual level factors and neighborhood deprivation scores and subsequently assigned a risk group. We hypothesized that levels of pro-inflammatory and anti-inflammatory cytokines in the first trimester differ by risk group and race. We found that Black females were more likely to be at risk for toxic stress and had lower median levels of IL-6 compared to White females. When stratified by risk group and race, low risk Black females had lower levels of IL-10 compared to low risk White females and high-risk Black females had lower levels of IL-6 compared to high-risk White females. Our results suggest the relationship between toxic stress and inflammatory cytokines is modified by race. It is our theory that exposure to repetitive stress due to racism and lifelong social disadvantage, results in desensitization of the stress pathway and a blunted adaptive response to future stressors. Understanding the mechanisms involved in the disparities in preterm birth are of the upmost public health significance.

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Preface

This manuscript would not be possible without the expertise and guidance from Dr. Janet Catov, Dr. Dara Mendez, and Dr. Toby Yanowitz. You all have made me a better researcher and clinician as a result of this project.

I would be remiss if I did not acknowledge my personal support system who has been instrumental throughout the completion of my masters curriculum. To my wonderful husband, Dr. Chigozirim Ekeke, thank you for being present every step of the way giving me critical feedback, encouraging words, or a warm embrace. To my mother, Denise Kingsberry, thank you for never questioning my drive and motivating me to always dream big. My success is a reflection of your hard work and unconditional love.

1.0 Background

1.1 Epidemiology of Preterm Birth

Preterm birth(PTB) is defined as birth before 37 weeks of pregnancy and affects approximately 1 out of 10 infants in the United States^[1]. Over the past two decades, there have been slight fluctuations in the distribution of preterm births. In 2007, preterm birth rates had been decreasing secondary, at least in part, to a significant decline in births to teens and young child bearing people ^[2]. From 2007-2014, there was a 39.5% decrease in births to teens and an subsequent increase in births to parents aged 25 and older which translated to modest improvements in preterm birth rates ^[3]. Most recently, preterm birth rates have steadily increased each year since 2014^[4]. Although the increases in preterm birth rates from 9.57% to a peak of 10.2% in 2018 may appear to be marginally significant, this translates to approximately 25,000 more infants who are born prematurely. Although every race has seen increases in their preterm birth rates since 2014, Black parents are disproportionately affected and the mechanism behind this racial disparity is poorly understood.

1.2 What is Known about the Racial Disparity in Preterm Birth

There is a racial disparity in birth outcomes in the United States that disproportionately affects Black parents. Black parents are 1.5 times more likely to have a preterm birth compared to Non-Hispanic White parents. Review of the literature has identified several contributing factors,

but controlling for individual-level maternal risk factors, such as smoking, chronic medical conditions, access to prenatal care, and socioeconomic status does not fully explain the racial disparity seen in preterm births^[5-7]. To date, the major contributors of the racial disparity in preterm birth rates remain unclear.

It is hypothesized that the etiology of the widening racial disparity involves a combination of individual and ecologic risk factors. An individual's behavior affects the method in which one can access and interact with their environment and has a direct effect on their health. A framework has been proposed that involves microenvironmental and macroenvironmental factors shaping the likelihood of a uncomplicated, term delivery^[6]. Within this framework, individual risk for preterm birth can be altered by behaviors where individual choice is in effect, such as smoking, diet, or exercise. These constitute elements of one's microenvironment and are the major contributors to an individual's medical history. In contrast, macroenvironmental factors include exposure to racism, poverty, and neighborhood violence and tend to have more of an effect on an individual's health outcomes.

1.2.1 Microenvironmental Factors Do Not Fully Explain the Racial Disparity

Medical conditions such as diabetes and hypertension have been shown to be associated with preterm birth. In the context of persistent obesity in the U.S., the prevalence of pre-pregnancy diabetes and chronic hypertension have increased significantly in all races but Blacks have seen a steeper increase in prevalence and severity ^[8, 9]. Both diabetes and hypertension have been found to be independent risk factors for spontaneous preterm birth ^[9, 10]. It has been hypothesized that these disease states lead to maternal and placental vascular malperfusion and this pathway increases an individual's susceptibility to preterm birth ^[11]. Concurrently, chronic hypertension

increases risk for pre-eclampsia which in the setting of severe clinical features may lead to medically indicated preterm birth. Despite the increased risk for preterm birth with certain individual level risk factors, controlling for BMI, diabetes mellitus, pregnancy-induced hypertension and eclampsia does not explain the over-representation of Black parents in preterm birth rates [10, 12].

Previously, factors such as access to medical care and insurance status have mentioned as risk factors for preterm birth. Some studies have suggested that those with late or limited prenatal care have worse birth outcomes compared to those receiving prenatal care in the first trimester [13]. Black females have been reported to have lower prenatal care utilization rates in some studies [14]. In 2016, 82.3% of Non-Hispanic White females began prenatal care in the first trimester compared to 66.5% of Non-Hispanic African American females (Martin et al., 2018). This intentional underutilization of prenatal care has been hypothesized to be due in part to exposure to institutionalized racism and perceived bias when presenting to medical attention [13, 15, 16]. Later presentation to care may negatively impact maternal and infant outcomes, however recent trends suggest the gap in prenatal care utilization is rapidly closing between races[15]. Many subsequent studies suggest that early prenatal care initiation and equal access to prenatal care attendance fail to explain the racial disparity in preterm birth [7, 17]. When comparing child-bearing people with comparable private pay health insurance and among child-bearing people in the military who are a low risk, healthy population with similar health plans, a differential risk for preterm birth by race is still present [7, 18]. Despite controlling for medical risk factors, severity of disease, and equal levels of PNC utilization, Black females still have higher rates of adverse birth outcomes compared to White parents [19, 20].

1.2.2 Social Risk Factors and Preterm Birth

In the addition to an individual's medical risk factors, social factors must also be considered as a significant influence on their personal health. Socioeconomic status has often been considered a major contributor to the racial disparity in preterm birth. Education attainment is one method to improve one's social position in society and access to resources. For Black parents, increases in maternal education attainment and income levels appear to not be as protective compared to other races. Racial disparities in preterm birth rates widen as education and income levels increase [21-23]. In low income groups, there is a similarly high rate of preterm births between races, but within the most socioeconomically advantaged group, there is a significant Black-White disparity in preterm birth rates. Well-educated Black females have higher preterm birth rates compared to White females of the same or lower educational status [23]. This alarming trend implores us to closely examine the unique experience of Black parents in the U.S. that may have a negative impact on their health outcomes.

Despite adequate access to medical care, there is a growing body of evidence that the quality of medical care Black people receive is often different than other races [24, 25]. Research has pinpointed the combination of the structural racism of the health system as well as the unconscious bias of health professionals to be responsible for the disparity in adverse birth outcomes[26-28]. Black inferiority attitudes, lack of empathy, and discriminatory hospital practices all contribute to the inequity of care received by Black people [24, 29]. As a result, stress has been posited as an intervening pathway between racism and preterm birth [30]. Chronic exposure to racism is not the only determinant of poor health in Black female but it's pervasive presence in society can alter and transform other social factors and can exacerbate the negative effects of other risk factors for health [24, 30].

1.3 Weathering and the Stress Pathway

The weathering hypothesis has been considered in an attempt to describe the cumulative effect of negative exposures on birth outcomes. Weathering is the theory that the health of Black people slowly deteriorates in early adult years as a result of cumulative negative stressors and socioeconomic disadvantage and thus increases their risk of adverse birth outcomes. Geronimus has studied the complex relationship between maternal age and preterm birth to highlight this effect. When looking at age-specific preterm birth rates, the decline in preterm birth rates seen by a decrease in teen pregnancies has been offset by a subsequent increase in preterm birth rates associated with an increase in births to parents > 30 years old and with increased use of assisted reproductive techniques [1, 31]. Maternal age has been found to be associated with preterm birth, but the relationship is multi-dimensional and modified by race. Traditionally, young maternal age and teen pregnancy have been considered to be a high-risk period for preterm birth and as parents progress into their 20s and early 30s, the risk for preterm birth decreases in this “prime child-bearing period”. For Caucasian parents, it remains true that younger maternal age is a risk factor for preterm birth. Conversely, according to the weathering hypothesis, age over 15 years in Black parents is associated with increased odds of low birth weight and preterm birth [32]. Black infants with teen parents appear to have a survival advantage compared to infants with older parents and this has been thought to be due to cumulative negative exposures that becomes detrimental to an individual’s health[32, 33]. The work done in 1992 has been further supported by additional studies and remains relevant in present discussions on the racial disparity in preterm birth and the physiologic effects in child-bearing people [33, 34].

1.3.1 Chronic Stress and Allostatic Load

Allostatic load is an increasingly hypothesized biological mechanism through which the differential exposure to adversity in the social and physical environment put African American parents at higher risk for preterm delivery relative to white parents [35, 36]. The concept of allostatic load refers to the physiologic “wear and tear” on the body as a response to repeated stress. Previous research has focused on identifying the critical point in which repetitive exposure to stressors results in physiologic changes and it has been suggested that critical points may be different for different races and directly relates to the concept of weathering[37, 38]. In 2006, Geronimus examined data from the National Health and Nutrition Examination Survey (NHANES) which assessed cumulative risk scores in adults aged 18-64 years in order to demonstrate that the weathering hypothesis is applicable outside of the confines of pregnancy. Each participant was assigned a cumulative allostatic load score based on the number of biomarkers in the metabolic and inflammatory domain that were noted to be in the highest quartile(>75%ile). Results showed Blacks had higher mean allostatic load scores compared to Whites, and Black females were the highest risk group overall [39]. This significantly elevated risk has been attributed to a “double hit” phenomenon due to social disadvantage from gender as well as race and echoes the sentiments of the weathering hypothesis.

1.3.2 The Relationship Between Weathering and Chronic Stress

While weathering is used to describe the cumulative effect of socioeconomic disadvantage, it can often be characterized by chronic stress that becomes toxic to an individual’s health. The concept of toxic stress has been brought into the discussion on adverse birth outcomes [40, 41].

Despite its negative connotation in society, all stress is not toxic and detrimental to an individual's overall well-being. There are varying levels of severity of stress which directly correlate to the degree of physiologic dysregulation. Positive stress refers to outside influences that lead to brief elevations in one's heart rate and mild elevations in stress hormones such as cortisol and epinephrine^[42]. This is often referred to as a fight or flight response. A defining characteristic of positive stress is once the stressor has been removed, the physiologic effects return to baseline. Tolerable stress represents an increase in severity in the description of stressors. Tolerable stress is characterized by a more significant response to intense stress that results in sustained elevations in stress hormones^[42, 43]. Although the stress response is more prolonged, it can slowly return to baseline weeks to months after the stressor is removed in the setting of supportive relationships and coping mechanisms.

1.4 Toxic Stress

In contrast, toxic stress refers to chronic exposure to physical, emotional, and/or environmental stressors that results in biochemical changes in the absence protective factors ^[42]. Unfortunately, when chronic stress becomes toxic, there is a failure to normalize these biochemical changes even after the stressor has been removed. Stressors can be categorized as physical and/or emotional stress, such as inter-partner violence; environmental stress, such as area deprivation or neighborhood violence; or psychological stress in the context of discrimination and perceived bias. Each type of stressor can affect the body's stress response and may lead to overstimulation of the hypothalamic-pituitary(HPA) axis and subsequent alterations along the stress pathway which can have long-term effects on an individual's health ^[44].

1.4.1 Why Do We Care About Toxic Stress?

The brain is a vulnerable target for stress and glucocorticoids have been shown to target the hippocampus^[45, 46]. Subsequently, stress hormones produce maladaptive effects and structural remodeling on the hippocampus, amygdala, and prefrontal cortex resulting in alterations in behavioral and physiologic responses ^[45]. The hippocampus has a role in cessation of the hypothalamic-pituitary axis response to stress, and dysfunction or atrophy of the hippocampus fails to shut off the HPA response which results in prolonged activation ^[47, 48]. In animal models, allostatic overload from chronic stress causes atrophy of neurons in the hippocampus and areas of the brain responsible for memory, executive function and selective attention ^[46, 49]. The “glucocorticoid cascade hypothesis” of stress and aging support this model. A study by Lupien et al. noted that annual increases in salivary cortisol over a 5-year period predicted hippocampal volume ^[50]. This demonstrates that repetitive stress does have an incremental negative effect on brain health. In addition to changes in the brain, toxic stress has also been hypothesized to lead to epigenetic changes, specifically telomere shortening and alterations in mitochondrial DNA ^[42, 43].

Lastly, it is important to better understand the effects of toxic stress because of its effect on the immune system. Toxic stress has been thought to activate the pro-inflammatory pathways which has long term impact on immune cell dysfunction with many downstream effects, including being implicated in preterm birth ^[42]. Pro-inflammatory and anti-inflammatory cytokines regulate each other to maintain physiologic homeostasis but these cytokines are also regulated by glucocorticoids and catecholamines. Catecholamines generally increase pro-inflammatory cytokine production, while glucocorticoids are known to inhibit. In regards to pregnancy, toxic prenatal stress has been postulated to be a factor in causing preterm birth, as well as full-term birth with low birth weight via an inflammatory pathway ^[51]. Females in the preconception period are

subjected to the daily stress of their lives, including distress related to poverty, domestic abuse, chronic medical conditions, and environmental stress. During pregnancy, this distress is compounded with stress surrounding the health of their infants and the obstacles that may prevent an uncomplicated term delivery. This cumulative stress may result in activation of the HPA axis and pro-inflammatory pathways which subsequently leads to placental inflammation and increased risk of preterm birth [52].

1.4.2 Who Is at Risk for Toxic Stress?

These studies highlight the broad implications of toxic stress and its wide range of effects on an individual's health status [42]. Based on previous literature, parents who are young, not married, have lower education status, and poor physical and mental health are at higher risk for stress [41, 53]. There are several other risk factors that have been identified including: poverty, racism, family violence, depression, and environmental factors that also contribute [41, 53]. All of these factors in a susceptible host have the potential to have negative implications on an individual's health.

1.5 Environmental Stress can also be Toxic

It is not just personally mediated stress that can be harmful. One's environment also plays a role in stress. Environmental deprivation is an important component of stress that accurately captures multiple social indicators of a group and has also been shown to be an independent risk factor for adverse birth outcomes [43, 49, 53]. Neighborhood-level stressors such as poverty, crime,

and racial composition have been shown to increase the risk of preterm birth [54]. Specifically, neighborhoods with high rates of poverty and racial composition of black residents are typically segregated and systematically isolated from opportunities and resources. There is a higher risk of preterm birth among residents of these neighborhoods for both Black and White parents. In addition to the cumulative negative effects of neighborhood factors, segregation into deprived neighborhoods as a result of institutionalized racism is a potential source of additional stress for parents [30, 53]. This supports the notion that environmental stressors should be considered when studying adverse birth outcomes.

Previous research has established that a number of social indicators tend to cluster at the neighborhood level, including the concentration of multiple markers of economic disadvantage. In a study conducted by Messer, birth record and crime report data were merged with census tract variables to describe birth trends based on geographic cohesion in census block groups[55]. They found non-Hispanic Black parents were more likely than non-Hispanic White parents to deliver preterm(12.8% versus 6.7%), live in economically deprived block groups (42.2% versus 19.3% in the highest deprivation quartile), and experience more crime (32.0% versus 3.8% in the highest violent-crime-rate quartile[56].

In addition to the characteristics of one's environment, an individual's negative perceptions of their environment also contribute to their health. In a community based assessment project examining the attitudes of females in their second trimester, perceived stress was reported more frequently among residents with a less hospitable residential environment, characterized by higher vacancy rates, more violent crime, more housing damage and property disorder[57]. In addition to the stress of being a resident of a deprived neighborhood, through isolation, the environment also can contribute to psychological stress. Lack of social support and use of avoidance coping

mechanisms have been related to higher levels of depressive symptoms in a study of pregnant, minorities [58]. Data from the 2001-2002 National Epidemiologic survey on Alcohol and Related Conditions showed that Black females were more likely to report major depression compared to non-Hispanic White females [59]. The increased risk of depressive symptoms has important implications for maternal wellness and their birth outcomes. Females with higher depression scale scores have been found to have increased risk for preterm birth, even after controlling for other sociodemographic and obstetric factors[60, 61]. It has been hypothesized that cortisol and other stress hormones are increased in major depression resulting in an enhanced release of placental corticotropin-releasing hormone(CRH), which plays a role in triggering the onset of labor. When considering the growing body of evidence, it seems clear there is a strong link between environmental variables, psychological stress and preterm birth.

1.5.1 Area Deprivation and Adverse Birth Outcomes

Due to heterogeneity in study designs, it remains unclear which environmental variables appropriately capture disadvantage and are most influential in assessing risk for stress and adverse birth outcomes. There are other established area-level indices that have been used to compare deprivation effects across geographic units[62, 63]. One particular index, the Messer index, was developed as a cumulative indicator of area deprivation and has been used commonly in previous studies on maternal child health. In a study conducted in 2006, principal component analysis(PCA) was performed on 20 census level variables across multiple domains, including education, income, education, employment, housing, and occupation. As a result of the PCA, 8 census tract variables were retained for the index. Now referred to as the Messer index, it is comprised of the following eight census variables: percent of males in management and professional occupations, percent of

crowded housing, percent of households in poverty, percent of female headed households with dependents, percent of households on public assistance and households earning <\$30,000 per year estimating poverty, percent earning less than a high school education, and the percent unemployed which all contribute approximately equally to deprivation across multiple settings. Compared to the other area-level indices that are available, the Messer index has the benefit of using easily accessible U.S. Census data that can be generalized across broad geographic units. This particular index has been particularly useful in subsequent studies on the effect of area deprivation on maternal and infant health because it has been found to be associated with the unadjusted prevalence of preterm birth in non-Hispanic White and non-Hispanic Black females [55]. With many potential sources for stress, the pathway to preterm birth is complex. Taking multiple levels of stress together, it is still unclear what the cumulative effect of physical, environmental, and psychological stress is on preterm birth and how we quantify that effect?

2.0 Measuring Toxic Stress

Due to the important implications of identifying those exposed to toxic stress, it is imperative that we have a valid measurement. Traditionally, self-report has been used as the primary method for assessing those exposed to stress. The Perceived Stress Scale(PSS) and the Stressful Life Events Inventory(SLEI) are two valid instruments that assess the degree to which life events are perceived as stressful. There have been several studies that have suggested this self-report system does accurately capture the degree of stress experienced [64]. Other studies have suggested that self-report is subject to bias and may be underreporting individuals significantly affected by stress due to their unwillingness to disclose [65]. To date, it remains unclear if self-report measures accurately reflect the biologic response to stress or if the use of biomarkers should be employed.

2.1 Use of Cytokines as Biomarkers

Biochemical markers, such as cytokine levels, can be used as an objective measure for toxic stress [37]. It is known that pro-inflammatory and anti-inflammatory cytokines regulate each other to maintain physiologic homeostasis. It is theorized that chronic exposure to stress disrupts that homeostasis and leads to inflammatory dysregulation. For example, Masho et. al performed a study of 231 expectant parents who were assessed at their first prenatal visit. They were given either the Perceived Stress Scale or the Stressful Life Events Inventory and a salivary cortisol level was obtained. The goal of the study was to examine how the relationship between perinatal stress

and preterm birth differs by self-identified race but secondarily they were also able to confirm stress scale scores with levels of stress hormone. Based on their study, stress measures using the PSS and SLEI were not significantly associated with preterm birth, but salivary cortisol levels were associated. For every 1µg/dL increase in cortisol level, the odds of preterm birth increased by 26% (29% in Black females). This suggests that despite the subjects experiencing a significant degree of perinatal stress, self-report measures may underestimate its severity or biologic response and thus objective biomarker measures should be explored. Although useful, practical use of cortisol levels as a proxy for stress is limited by fluctuating levels based on time of day.

Previous studies have identified a variety of other biomarkers as a marker of chronic stress with a focus on the inflammatory pathway. DHEA, pro-inflammatory cytokines, CRH, and TNF-alpha have all been implicated to be part of the biophysical dysregulation in response to stress [37, 38, 66]. Prenatal maternal stress can be characterized by a cumulative stress indicator which has been shown to not only affect the mothers but also the immune system of infants. After antigenic microbial stimulation, prenatal stress was associated with higher levels of IL- 8 and IL-13, and TNF-alpha resulting in alterations in innate and adaptive immune response in the cord blood of infants which suggests that the in-utero environment leads to measurable biochemical changes in infants [20, 67]. Pregnancy is a physiological state that requires special consideration when examining the delicate balance between proinflammatory and anti-inflammatory states. Previous studies have noted that even in otherwise normal pregnancies, high levels of stress with low social support was associated with low levels of the anti-inflammatory cytokine, IL-10, early in pregnancy and higher levels of the pro-inflammatory cytokine, IL-6, later in pregnancy but this relationship between inflammation and pregnancy is dynamic[68]. Assessment in the first trimester

likely reflects chronic stress prior to pregnancy while assessment of cytokines later provides a better picture of pregnancy-related stress.

2.1.1 Inflammation and Preterm Birth

When looking later in pregnancy, infection and inflammation are known risk factors for prematurity. In some studies, at least 25% of all preterm birth cases have an identified infection^[19, 69, 70]. In extreme prematurity, as high as 79% of patients tested positive for infection, which suggests infection-related inflammation may also contribute to the timing of parturition. Commonly, infectious pathogens, have been directly correlated with increased production of intra-amniotic pro-inflammatory cytokines, particularly IL-6 ^[71]. In the absence of a well-documented infectious trigger, chronic inflammatory and immunologic abnormalities have also been associated with preterm birth^[72]. In addition to IL-1, TNF-alpha, and IL-6 being implicated in the onset of PTB, polymorphisms in proinflammatory and anti-inflammatory genes has also been associated with preterm birth^[73-75]Anti-inflammatory cytokines, such as IL-10, have been found to be highly expressed in the uterus and placenta and implicated in silencing of pro-inflammatory cytokines(TNF-alpha, IL-6, and IL-1). However, despite evidence of colonization, the mechanism behind the pathway to preterm birth remains unclear given not every mother with an infection goes into preterm labor.

2.1.2 Gaps in the Literature on Stress and Inflammation

Through review of the literature, it has been established that prenatal stress in the form of physical, environmental, and psychosocial stress has a negative impact on preterm birth rates.

Literature also supports that inflammation has a role in preterm birth. Our study sets out to more closely examine the relationship between toxic stress and inflammation(Figure 1). Previous studies have primarily focused on the effects of singular risk factors. There is currently sparse data on the cumulative effect of personal and environmental factors on the stress pathway via dysregulation of inflammation. The main purpose of our study is to examine if those at high risk for toxic stress, as characterized by high risk individual and environmental factors, also have higher median levels of pro-inflammatory cytokines compared to low risk in the first trimester of pregnancy. The goal is to evaluate exposure to varying levels of chronic stress so first trimester blood samples will be utilized in order to reduce confounding with pregnancy-related states related to infection and inflammation. Conclusions from this analysis would serve as a first step in closing the gaps in knowledge in the relationship between environmental stress, high risk maternal characteristics, and inflammation.

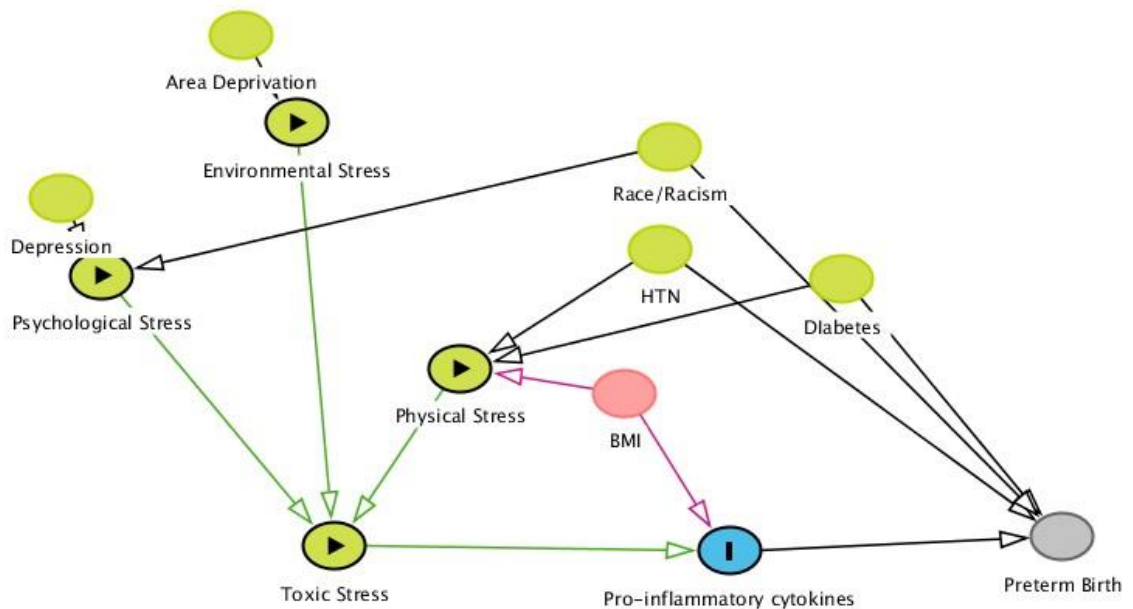


Figure 1. Directed Acyclic graph (DAG) of Relationship between Toxic Stress, Inflammation and Preterm Birth

3.0 Study Design

This was a cross-sectional study conducted in Pittsburgh, PA and was approved by the Institutional Review Board of University of Pittsburgh. We enrolled 37,474 participants in the MOMI database aged 15-45 years old who delivered live, singleton infants during the time period of interest (2007-2013). MOMI is an electronic database of maternal and infant variables from all deliveries at Magee Women's Hospital. In addition to clinical information, the database is associated with a biobank which holds residual blood samples collected from the first and second trimesters of pregnancy. Approximately 20% of mothers delivering at Magee-Women's Hospital have specimens in the MOMI Biobank. The mothers that contribute are a diverse group and demographically represent all deliveries at Magee Women's Hospital. For the purposes of our study, mothers were excluded if they did not have complete data for any of the pertinent risk factors, infant gestational age, or residential address. If a participant had multiple deliveries in the time period of interest, the first delivery was used for analysis and the remaining were excluded. In our study, our overall population included 66,792 participants in the MOMI database aged 15-45 years old who delivered live, singleton infants at Magee Women's Hospital during 2007-2013. 43% (N=29,318) were excluded from analysis due to unspecified race, incomplete data for clinical risk factors, infant outcomes, geographic data, or duplicate entries per participant's ID for subsequent deliveries. This left a remaining sample of N=37,474 participants.

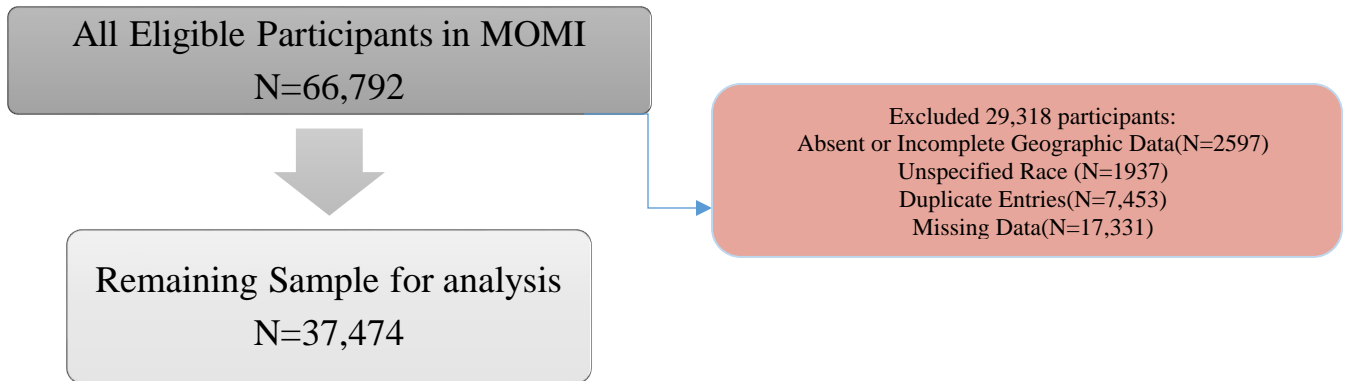


Figure 2. Sample Selection Process for Enrollment

3.1 Description of variables

Individual-level Variables

Maternal exposures of interest were collected: maternal education, BMI, history of chronic illness (defined as hypertension or diabetes), smoking status, marital status, history of depression, and history of substance abuse. Infant variables of interest included birth weight and gestational age. Covariates of maternal age and race were also collected to be included in statistical models. Residential address identified by each mother at time of delivery was also extracted. Address was used to assign participants into their corresponding census tracts from which we could assess the degree of neighborhood deprivation. Data was limited to 2007-2013 due to availability of geocoded data.

Census Data

Socio-economic information from the United States Census Bureau was collected by census tract using 2009-2013 American Community Survey(ACS) 5-Year Estimates. Census tracts were used as a rough equivalent to a neighborhood. Census tracts usually encompass approximately 2,500 to 8,000 people and have been noted to be small, relatively permanent groupings within a county.

Data at the census tract level was used due to stability in estimates and the fairly homogenous unit of analysis with respect to socio-demographic characteristics and living conditions^[55]. Information for the 8 census level variables that comprise the Messer Index were obtained: percent of males in management and professional occupations, percent of crowded housing, percent of households in poverty, percent of female headed households with dependents, percent of households on public assistance and households earning <\$30,000 per year estimating poverty, percent earning less than a high school education, and the percent unemployed.

Area Deprivation Index

Messer index was utilized because it has been used as a composite measure of area deprivation in previous studies on maternal and infant outcomes ^[55]. Using census tract data regarding their neighborhood, each woman was assigned a Messer Area Deprivation Index (ADI) score. Tertiles of the continuous neighborhood deprivation index score were created with higher percentiles indicating a higher degree of area deprivation(Figure 4). The tertiles were defined as low deprivation(<33%ile), moderate(33-66%ile), high deprivation(>66%ile).

3.2 Risk Stratification of Cohort

In the investigative portion of the study, risk stratification of the entire cohort was performed prior to obtaining a simple random sample for further analysis. Risk groups were assigned for the entire cohort as low, moderate, and high risk for toxic stress which was assigned by the principal investigator based on the frequency of stressors/risk factors present. Risk factors for toxic stress included individual level characteristics such as low maternal education (HS diploma or less), BMI >25, smoking status of current smoker, marital status of unmarried, history

of chronic illness, history of depression, and history of substance abuse, in addition to presence of significant area deprivation, which is defined as having an area deprivation (ADI) score in the highest tertile (>66 percentile). Each risk factor was converted into a dichotomous variable and assigned zero if absent and one if present.

The distribution of cumulative risk factors per participant was assessed using Kolmogorov-Smirnov test of normality in the entire cohort(N=37,474). (Figure 5). Based on the distribution of total number of risk factors per subject, risk groups for toxic stress were formed. For the purposes of our study, the low risk group was defined as participants with 0-1 risk factors. Moderate or intermediate risk for toxic stress was defined as participants with 2 risk factors. High risk for toxic stress was defined as participants with 3 or more risk factors. There were 39.5%(N=14,818) in the low risk group with 0-1 risk factors, 42.3%(N=15,849) with 2 risk factors, and 18.2%(N=6,807) in the high-risk group with 3 or more risk factors. Once the entire cohort was risk stratified, a random sample was taken of 100 Black mothers (40 high risk, 20 medium risk, 40 low risk) and 100 White mothers(40 high risk, 20 medium risk, 40 low risk) for further analysis.

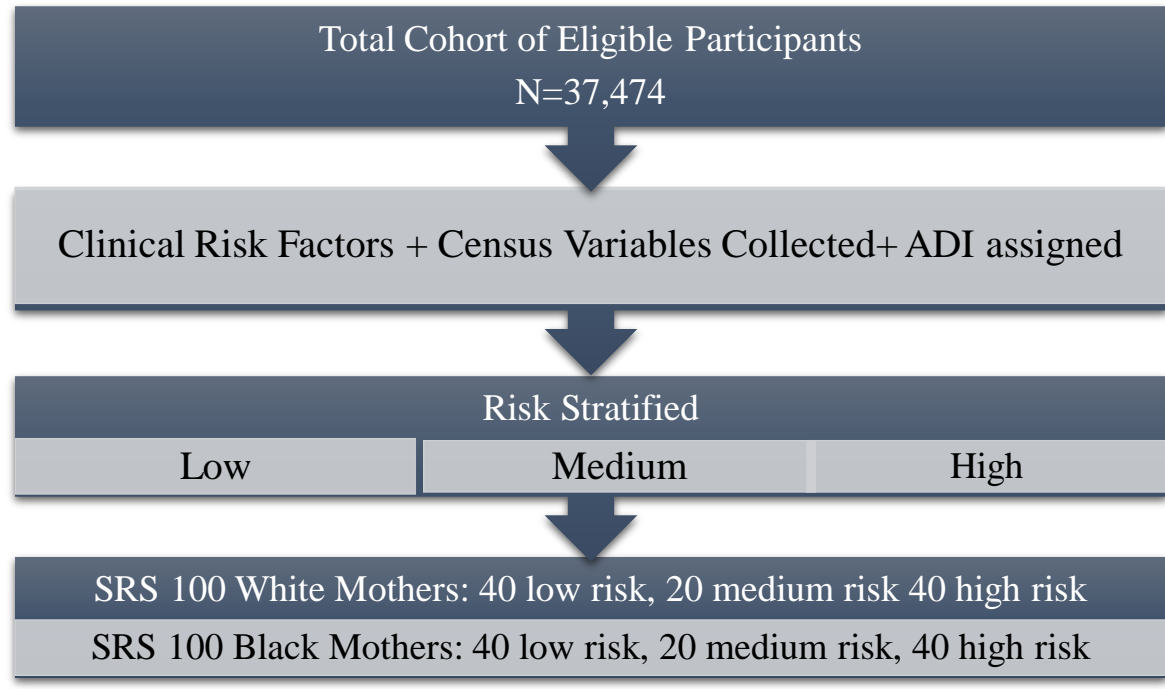


Figure 3. Random Sample Selection Process Map

3.3 Cytokine analysis

Maternal blood specimens were collected in the first trimester of pregnancy (mean gestational age 12.5 weeks), aliquoted and centrifuged immediately after collection. Specimens have been stored long term in the Magee Women’s Research Institute (Pittsburgh, PA) ultra-low freezer. Once thawed, a panel of inflammatory markers (IL-6, IL-8, IL-10, IL-13, TNF-alpha) was run using multiplex fluorescent bead-based assay (Human High Sensitivity T Cell Magnetic Bead Panel, 96-Well Plate Assay) in the Luminex Core Laboratory. The observed concentration (pg/mL) of each analyte for each sample was calculated. Any concentration that was noted to be out of range(OOR) indicated the level was either too high or too low to detect. For values noted too low to detect, a value at the lower limit of detection was assigned. For values noted too high

to detect, a value at the upper limit of detection was assigned. There were approximately 2%(4/200) that were noted to be out of range.

The distribution of each cytokine was assessed for normality using the Kolmogorov-Smirnov tests for normality. IL-6 was not normally distributed($p<0.0001$) and had a median(Q1,Q3) level of 10.1 (4.0, 23.3). IL-8 was not normally distributed($p<0.0001$) had a median(Q1,Q3) level of 12.6 (8.0, 31.9). IL-10($p<0.0001$) was not normally distributed had a median(Q1,Q3) level of 34.9 (24.8, 34.9). IL-13($p<0.0001$) was not normally distributed had a median(Q1,Q3) level of 7.9 (5.4, 14.5). TNF-alpha($p<0.0001$) was not normally distributed with a median of 8.1 (6.3, 10.6). Spearman correlation coefficients were calculated to assess each cytokine's relationship with each other(Table 4). Since none of the cytokines had a normal distribution, nonparametric tests were performed for all subsequent analysis.

3.4 Statistical Analysis

The primary aim was to determine if the participants identified as high risk for toxic stress had higher median cytokine levels compared to medium and low risk women. Median cytokine levels were calculated for each risk group. Wilcoxon rank sum tests performed to assess for differences in median cytokine levels stratified by risk group alone and race alone. Stratified analyses was performed by risk group and race to assess for differences in median cytokine levels by risk group in A)White mothers B) Black mothers C) Difference by race within the high risk group D) Difference by race within the moderate risk group and E) Difference by race within the low risk group. Dichotomous variables were created indicating those cytokines in the highest quartile(>75%ile). Kruskal Wallis tests were conducted to assess for baseline differences between

those in the highest quartile for each cytokine and those who were not in the highest quartile. Secondary analysis was performed to assess for differences in median cytokine levels stratified by race. Characteristics were compared between risk groups, using spearman's correlation coefficient for continuous variables and Kruskal-Wallis tests for categorical variables. Log binomial regression models performed examining the relationship between those in the highest quartile(>75%ile) for each cytokine and risk group while adjusting for race and age. The statistical package for analysis was SAS software, version 9.4 (SAS Institute Inc., Cary, NC, US).

4.0 Results

Within the entire cohort (N=37,424), there were differences in the baseline characteristics by race (Table 1). Black participants were younger(24.8 vs 29.5 years, $p < 0.0001$), had higher pre-pregnancy BMI(27.4 vs 25.4 kg/m², $P < 0.0001$), and were more likely to have hypertension(9.2% vs 6.4%, $p < 0.0001$) and depression(11.1% vs 8.8%, $p < 0.0001$) compared to White participants. They were less likely to be married(11.9% vs 66.7%, $p < 0.001$) and less likely to smoke(14.5% vs 19.8%, $p < 0.0001$) compared to White participants. Regarding birth outcomes, Black participants had higher preterm birth rates(12.2% vs 8.7%, $p < 0.0001$), higher rates of small for gestational age(<10thile for gestation) (16% vs 7.8%, $p < 0.0001$) and their infants had lower birth weights(3097g vs 3349g, $p < 0.0001$). As shown in Table 2, when comparing the preterm versus term groups, those that had a preterm delivery were more likely to be unmarried(43.9% vs 41.4%, $p = 0.0043$), have a depression history(10.0% vs 8.8%, $p = 0.014$), hypertension history(9.8% vs 6.1%, $p < 0.0001$)and be a smoker(17.5% vs 14.4%, $p < 0.0001$) and less likely have less than or equal to HS diploma(41.3% vs 43.6%, $p = 0.008$).

For the sample stratified by risk groups(Table 3), the high risk group was more likely to smoke(34.85% versus 5.89%, $p < 0.0001$), be unmarried(57.14% versus 10.59%, $p < 0.0001$), have a high school diploma or less(54.44% versus 9.41%, $p < 0.0001$), have a history of hypertension(16.90% versus 5.89%, $p < 0.009$), depression(23.94% versus 1.15%, $p < 0.0001$), and preterm birth(15.58% versus 7.06%, $p = 0.047$) compared to the low risk group. There was no statistically significant difference in proportion of participants that were overweight(BMI>25) or had history of diabetes. In the high-risk group, 53/77(68.8%) live in a deprived area compared to

15/39(38.4%) in the moderate risk group and 4/85(4.7%) in the low risk group that lived in a deprived area with a p-value of < 0.0001.

The results of the Spearman correlation coefficient cytokine matrix are depicted in Table 4. IL-6 was strongly correlated with IL-13 (R= 0.828, p-value <0.001) and weakly correlated with IL-8(R=0.381, p-value <0.0001) and IL-10(R=0.578, p-value <0.0001). None of the other cytokines(IL-8, IL-10, IL-13, TNF-alpha) showed any statistically significant correlations with each other. The relationship between each cytokine and ADI was assessed and IL-6 had a weak negative correlation with area deprivation (R=-0.155, p-value 0.029).

Univariate analysis was performed to assess for differences in ADI score by clinical risk factors. Participants that were not in a committed relationship had higher median ADI scores 1.31(-0.41, 4.17) compared to participants not in a committed relationship with median ADI score of -0.356(-1.47, 2.42), indicating more area deprivation(p-value 0.0005). The participants with high school diploma or less had had higher median ADI scores of 0.987 (-0.071, 3.96) compared to women with greater than a high school diploma with ADI score of -0.033 (-1.34, 3.17), indicated more area deprivation(p-value 0.021). There were no statistically significant differences in ADI based on smoking status, depression, hypertension, diabetes, or BMI. The participants in the most deprived area(ADI> 66%ile) had lower median IL-6 levels compared to those in the moderately deprived and least deprived groups.

When stratified by risk group, there were no statistically significant differences in median levels of IL-6, IL-8, IL-10, IL-13, or TNF-alpha(Table 5). When stratified by race, Black participants were found to have lower median IL-6 levels (8.80 vs 10.56, p=0.037) compared to White participants(Table 6). Median IL-8, IL-10, IL-13, TNF-alpha levels were not statistically significantly different between Black participants and White participants. When stratified by race

and risk group, there were differences between median cytokine levels observed (Table 7). High risk Black participants had lower IL-6 levels (7.85 vs 13.86, $p=0.054$) and compared to high risk White participants. Low risk Black participants had lower IL-10 levels (31.47 vs 42.31, $p=0.018$) compared to low risk White participants. There were statistically significant differences between medium risk Black and White participants. When examining those at the highest quartile for each cytokine compared to those who were not, there was no significant differences in rates of clinical risk factors with the exception of hypertension and elevated IL-6. The participants with IL-6 levels in the highest quartile had higher rates of hypertension (20.0% versus 7.8%) compared to those who were not in the highest quartile ($p=0.022$). IL-8 and IL-13 had hypertension rates of 16.7% ($p=0.121$) and 17.8% ($p=0.078$), respectively which were higher than the hypertension rate of the subsample (20/200, 10.0%) but this was not significantly different by highest quartile status. Similarly, for preterm birth rates, IL-6 and IL-13 had rates of 5.9% ($p=0.307$) and 6.0% ($p=0.334$), respectively, although there was no significant difference by highest quartile status.

The results of the log binomial regression model are displayed in Table 8. When adjusting for age and race, there was no statistically significant increased risk for being in the highest quartile for IL-6, IL-8, IL-10, IL-13, or TNF-alpha by risk group. There was a trend toward significance for Black participants in the highest quartile for IL-8 with $RR=1.22(0.71, 2.10)$ and TNF-alpha with $RR=0.71(0.43, 1.19)$.

5.0 Discussion

Our study emphasizes the importance of racism in the discussion of toxic stress. Based on our results, there were no appreciable differences in median cytokine levels between participants that were deemed high risk for toxic stress compared to moderate and low risk. When considering race, we found that Black participants had lower IL-6 levels compared to White participants. Additionally, the relationship between cytokines and risk group appears to be race dependent. For low risk pregnant females in the first trimester with less exposure to chronic stress, the anti-inflammatory pathway is suppressed with lower levels of IL-10 in Black females compared to White females. This suggests that even for participants with low levels of pre-pregnancy stress, there is still a White-Black difference that is left unexplained. We theorize that lifelong minority status and cumulative exposure to racism may alter the baseline inflammatory milieu for Black females. For high risk pregnant females in the first trimester, Black females similarly had lower IL-6 levels compared to high-risk White females (Figure 7). Although not statistically significant a similar trend is observed with IL-8 where as risk for toxic stress increased, cytokines levels decreased. These biochemical differences are important to note because they identify potential targets along the pathway from stress to preterm birth although future studies would need to be conducted to confirm that the biochemical changes seen in early in pregnancy in this study indeed are associated with birth outcomes later in pregnancy.

Based on previous studies, our original hypothesis was that participants that were at higher risk for toxic stress would experience activation of their pro-inflammatory cytokines and thus would have higher median levels, but our results introduce the notion that race-specific risk stratification is crucial to understanding any differences. We theorize that the unique experience

of being a Black female in America with repeated exposure to racism and sexism is a source of chronic stress that may result in decreased HPA axis reactivity and blunted responses to future stressors. This provides further context to the concept of toxic stress, highlighting the prevalence of immune system dysregulation and maladaptive future stress responses.

This theory is further supported by the differences in cytokine levels when our cohort was stratified by risk group and race. Even in the low risk group, Black participants had lower IL-10 levels compared to White participants(Figure 8). IL-10 is traditionally considered an anti-inflammatory cytokine and has been shown in previous studies to inhibit the synthesis of pro-inflammatory cytokines such as IFN- γ , IL-2, IL-3, TNF α and GM-CSF. With low levels of IL-10, there is an imbalance in the inflammatory homeostasis and a propensity for a pro-inflammatory state. Even in the absence of many risk factors for stress, Black participants in our study have a lower threshold for HPA axis desensitization outside of medical, sociodemographic, and environmental risk factors that are involved in repetitive activation of the stress response. It has been well documented that even the most socioeconomically advantaged group of Black females often cite workplace discrimination in the form of microaggressions, overt racism and sexism are a chronic source of stress [76, 77]. As individual risk factors accumulate in the high-risk group, Black females are still being exposed to race-based discrimination and may experience burnout of their pro-inflammatory pathway, thus leading to lower IL-6 levels compared to White females with similar individual risk factors.

IL-6 appears to play a significant role in the stress pathway. When psychologically stressed, the human body triggers release of IL-6 into the circulation as a result of stress hormones such as cortisol[78]. IL-6 has many functions with both anti-inflammatory and pro-inflammatory components. It is often an important mediator of the acute phase response, but it is unclear how

IL-6 levels change in response to chronic repetitive stress. Our current theory is that repetitive stress results in desensitization of the stress pathway and the receptors involved, so in the face a new stressor, one is unable to mount a full, robust stress response. With this mechanism, there would be a blunted inflammatory response where levels of IL-6 would be lower than expected. Our results provide further support of this phenomenon which has been previously documented in the literature. Previous studies have examined the stress response of adolescents who were being maltreated and exposed to chronic stress through bullying. When compared to the control group, maltreated/bullied child had lower cortisol levels in response to additional psychosocial stress^[79]. While several studies have reported similar effects of decreased HPA axis reactivity in response to chronic stress, our study extends current knowledge to include cytokines such as IL-6^[44, 80-83].

Cytokines are one method of assessing the physiologic effects of stress in an individual, but individual level effects do not give the full picture. Although there is considerable work to be done understanding the mechanisms underlying toxic stress, there have been several risk factors that have been found to be important. In addition to individual level risk factors such as hypertension, diabetes, and low education levels contributing to the disparities in preterm birth, other risk factors such as maternal age, access to care and area deprivation allude to the complexity of the issue. Given that many risk factors are correlated, and the differential effects of stressors that are often modified by environment, it is clear the conversation on disparities in stress and preterm birth goes beyond individual behaviors. Our study encourages us to closely examine the stress pathway with a race-conscious lens.

Disparities in preterm birth are likely not the result of the singular effect of any risk factor but how medical and environmental stressors in combination with lifelong exposure to racism, sexism, and disadvantaged social status lay the foundation for HPA axis dysregulation. Previous

studies have pointed to the activation of the inflammatory pathway as the mechanism behind the relationship between toxic stress and preterm birth. Pro-inflammatory cytokines are associated with uterine contraction and PTB and anti-inflammatory cytokines play a critical role in uterine quiescence during gestation^[69]. Similar to preterm birth, the mechanism underlying differential responses to stress by race also appears to be multi-factorial.

Our study emphasizes the importance of considering the individual in a greater social context. Area deprivation is a factor that encompasses multiple environmental stressors into one variable. Using the Messer index(ADI) as a risk factor for toxic stress helped to make more distinct risk groups. With the addition of ADI, there was a shift with a clear delineation between low, moderate, and high-risk groups. The majority of the high-risk group (68%) lived in the most deprived area(ADI> 66thile) compared to 4.7% in a deprived area from the low risk group. In addition to being influential in risk group delineation, ADI was also associated with biochemical changes. ADI was correlated with IL-6 in a slightly negative direction. Based on our results, as area deprivation increased, IL-6 levels decreased by 0.155. Although a modest effect, the negative correlation further supports our findings of a blunted HPA axis response in the setting of chronic stress.

One's exposure to environmental chaos, violence, and poverty is a durable component of environmental stress that throughout one's lifespan can be the source of multiple more acute stressors. Repetitive environmental stress, similar to other components of toxic stress, likely results in physiologic "wear and tear" on an individual, thus affecting their reactivity to future stressors. It is not surprising that of the group of clinical risk factors selected, marital status and maternal education attainment were also significantly associated with ADI scores because both of these factors are reflected to some degree in the census variables that make up the ADI and speak to an

individual's position in society and social environment. Highly educated parents, in addition to receiving a personal advantage due to their education status, also confer those benefits onto their household in the form of economic attainment^[21]. It stands to reason that neighborhoods are comprised of individuals with similar education status and economic attainment and via their environment they would be privileged or deprived of resources and health-promoting behaviors. Marital status, although considered an individual level variable, also speaks to the privilege of an individual. Both high maternal education and being in a committed relationship often implies there is some degree of social support which is crucial when discussing toxic stress.

Although our study confirmed the relationship between several individual risk factors and stress, it remains unclear which risk factors are most important in the relationship between stress and preterm birth. Our study extends our knowledge about the relationship between inflammation and stress to not only include elevations in pro-inflammatory cytokines but also in a susceptible host a blunted response in the pro-inflammatory pathway that affects the adaptive response of individuals to future stressors which in future studies may be able to be linked to preterm birth disparities.

Assessment of cytokine levels and clinically significant differences was limited due to the cross-sectional design of the study. The cytokine levels assessed in this study merely provide a snapshot of the dynamic complexity of biochemical changes associated with stress. It is possible pro-inflammatory and anti-inflammatory changes are different at different time points prior to pregnancy and through each trimester of pregnancy. For this reason, we are not able to draw any valid conclusions about what effect the observed biochemical effects have on birth outcomes, particularly preterm birth since first trimester samples were used and the primary goal of the study was to examine the relationship between stress and inflammation, not inflammation and preterm

birth. We identified two additional limitations of our study which may explain why we were unable to detect a difference in median cytokine levels in participants that were deemed high risk for toxic stress compared to moderate and low risk participants. First, small sample size significantly limited the power of our study and with larger numbers we may be able to more accurately assess the more subtle differences between risk groups and inflammation. Secondly, it is possible that due to the complexity of the relationship of risk factors, the use of a cumulative risk score is insufficient. Future studies are needed with sufficient power for multilevel modeling and subgroup analysis to further delineate which environmental and individual factors are the most significant contributors and how cytokine levels change closer to the time of delivery. This study provides preliminary insight into establishing the link between inflammatory changes associated with stress early in pregnancy, but future prospective studies would be helpful in confirming the proposed role of chronic stress and cytokines in the preterm birth pathway as levels change throughout each stage of pregnancy up until birth. Our study extends our knowledge about the relationship between inflammation and stress to not only include elevations in pro-inflammatory cytokines but also in a susceptible host a blunted response in the pro-inflammatory pathway that affects the adaptive response of individuals to future stressors which in future studies may be able to be linked to preterm birth disparities.

5.1 Future Implications

There is a growing body of research focused on defining stress and its associated implications but in the future, the focus should be on translating the existing knowledge into practical solutions. Despite the significant burden chronic stress has on an individual, there are

limited options available now to mitigate its effect. Since the brain is the central organ involved in the stress response, brain-centered interventions have been found to have a positive impact on stress reduction and building resilience. Brain-centered interventions involve lifestyle modifications and change in behaviors such as improving sleep quality, cultivating a positive outlook on life, and moderate physical activity. Although somewhat dependent on intrinsic motivators of an individual, there is a role for public policy and community empowerment of healthy lifestyles by programs encouraging smoking cessation, home or work incentive programs, or by building community recreational centers as a way to improve overall health. Although these programs often are also helpful in stress reduction, there has been limited effect on decreasing disparities in preterm birth rates by targeting individual behavior.

Another behavioral intervention that has been shown to be effective at mitigating stress is social support^[87]. Having regular social contacts with supportive friends, family, or health professionals who provide emotional and instrumental support has been shown to reduce the allostatic load score, which measures physiological markers related to chronic stress ^[88] and also has been shown to halt telomere shortening ^[89]. There is limited data currently on the effect that social support has on brain circuitry, but it has been documented to improve mood and overall mental health ^[90-92]. In respect to adverse birth outcomes, there is a growing body of literature promoting the benefits of group prenatal care by increasing social support. When compared to those that received standard prenatal care, parents that participated in group prenatal care had lower preterm birth rates and low income African American parents received the most benefit^[93]. If we are to decrease disparities, future interventions should target the most socially disadvantaged in order to effectively close the gap.

In conclusion, we know that toxic stress has short term and long-term implications on maternal and infant health. While previous literature suggested chronic stress resulting in prolonged activation of the inflammatory pathway, our study adds an interaction between race and risk factors for stress as a possible explanation for the racial disparities in pre-pregnancy stress. Future studies are needed to better understand the race-dependent biochemical differences associated with chronic stress and how the physiologic changes throughout pregnancy directly contribute to adverse birth outcomes.

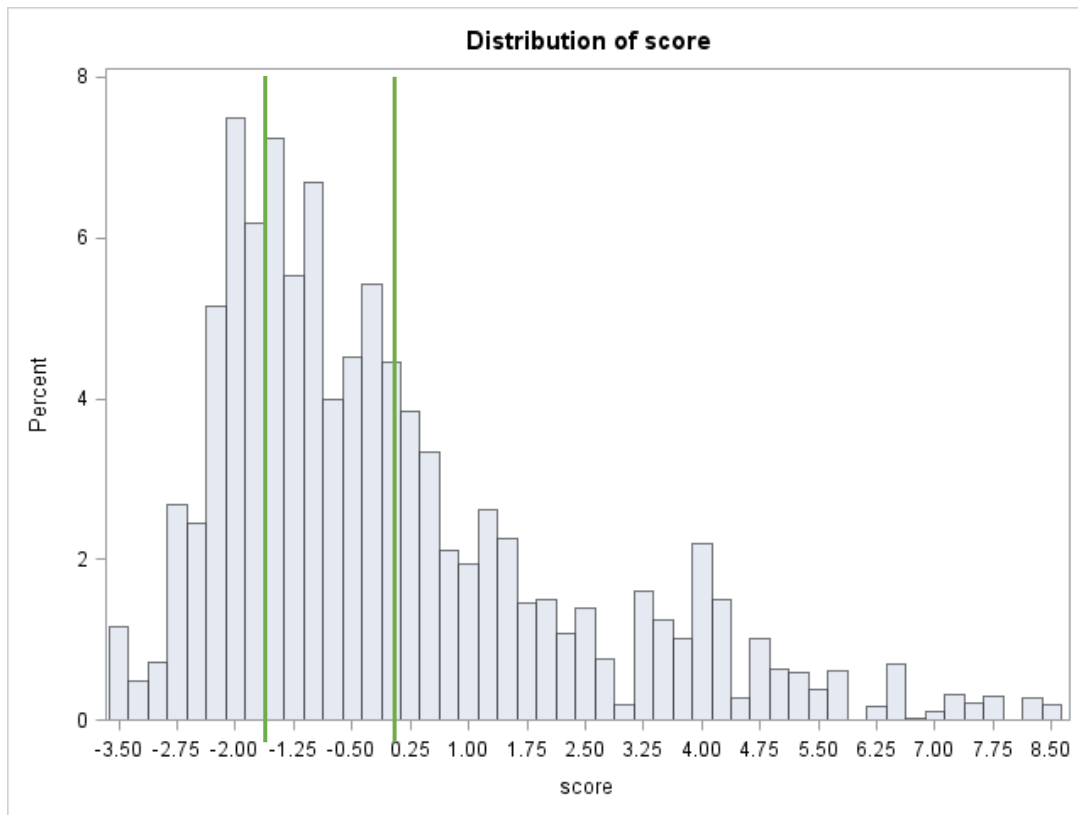
Appendix Tables and Figures

Table 1. Racial Differences in Baseline Characteristics of Entire Eligible Cohort (N=37, 474)

	White	Black	p-value
Teen	1113(3.9%)	1373(18.5%)	<0.0001
Preterm	2614(9.0%)	911 (12.2%)	<0.0001
SGA	5027(8.3%)	2694(16.4%)	<0.0001
Age	29.6(SD 5.4)	24.8(SD 5.7)	<0.0001
Hypertension	1861(6.4%)	683(9.2%)	<0.0001
Diabetes	1813(6.3%)	358(4.9%)	<0.0001
Smoking	3258(19.8%)	1160(14.5%)	<0.0001
Hx of Depression	2565(8.8%)	825(11.1%)	<0.0001
Marital Status (Committed, Married or Life Partner)	18,977(66.7%)	875(11.9%)	<0.0001
High School Diploma or less	9965(34.5%)	5726(77.1%)	<0.0001
Professional Degree	1679(5.8%)	77(1%)	<0.0001
Pre-pregnancy weight(kg)	68.6 (16.8)	73.7(20.2)	<0.0001
Weight at Delivery(kg)	83.6(SD 17)	87.6(SD 20.5)	<0.0001
BMI	25.4(SD 5.90)	27.4(SD 7.1)	<0.0001
Birth weight(g)	3349(SD 580)	3097(SD 636)	<0.0001

Table 2. Baseline Characteristics Stratified by Preterm versus Term

	Preterm N=3511	Term N=33, 209	P-value
BMI > 25	311(8.86%)	2875(8.68%)	0.729
Diabetes Hx	256(7.29%)	2159(6.5%)	0.086
Unmarried	1527(43.94%)	13,550(41.43%)	0.0043*
Hypertension Hx	344(9.80%)	2022(6.11%)	<0.0001*
<=HS diploma	13,684(41.33%)	1532(43.63%)	0.008*
Smoker	549(17.48%)	4270(14.44%)	<0.0001*
Depression Hx	352(10.03%)	2902(8.76%)	0.014*



ADI < -1.399
Least Deprived

ADI > 0.288
More Deprived

Figure 4. Distribution of Messer Area Deprivation Index (ADI) Scores of Entire Cohort (N=34, 747)

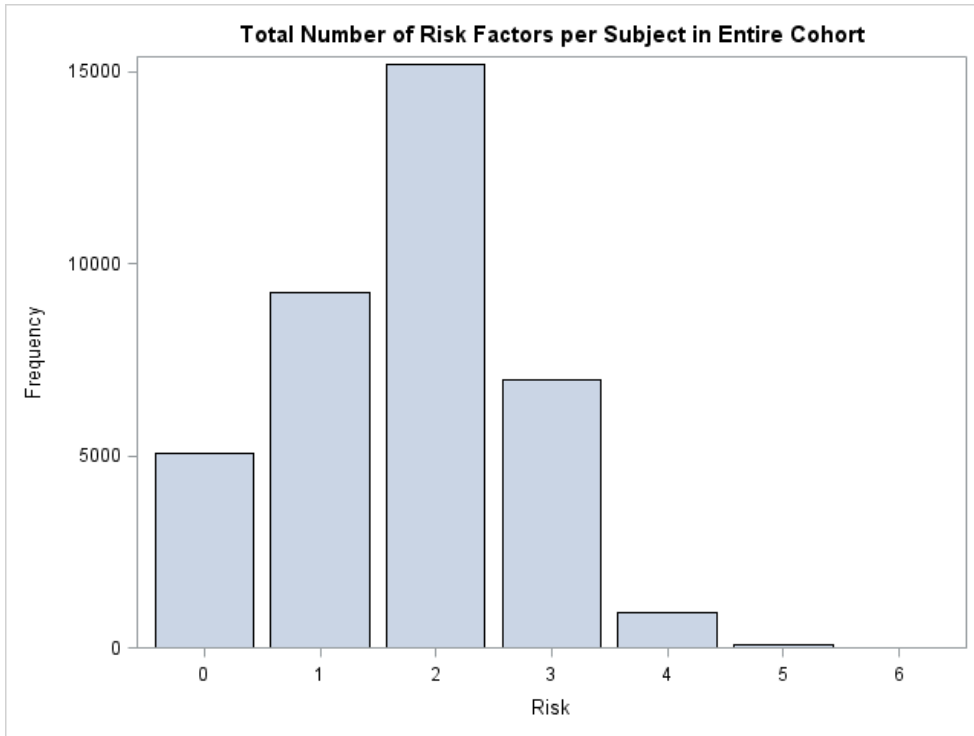


Figure 5. Distribution of Risk Factors for Entire Cohort (N=37,474)

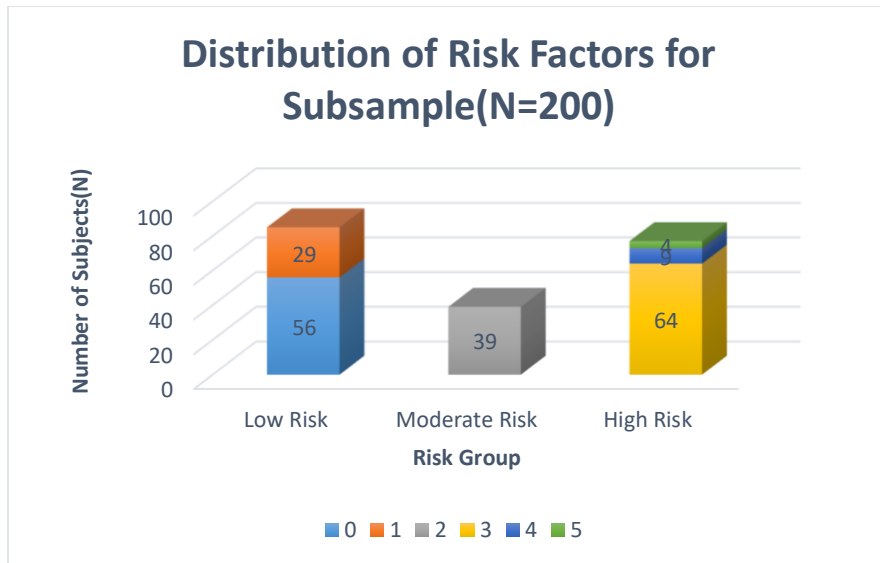


Figure 6. Distribution of Risk Factors for Subsample (N=200)

Table 3. Baseline Characteristics of Stratified Sample(N=200)

	Low Risk N=85	Medium Risk N=39	High Risk N=77	P-value
BMI > 25	3 (3.90%)	2 (5.13%)	4 (5.63%)	0.881
Hx of Diabetes	2 (2.29%)	5 (12.82%)	4 (5.63%)	0.0863
Unmarried	9 (10.59%)	25 (64.10%)	44 (57.14%)	<0.0001
Hx of Hypertension	5 (5.89%)	3 (7.69%)	12 (16.90%)	<0.009
<=HS diploma	8 (9.41%)	24 (61.54%)	42 (54.55%)	<0.0001
Smoker	5 (5.89%)	3 (8.82%)	23 (34.85%)	<0.0001
Hx of Depression	1 (1.15%)	2(5.13%)	17 (23.94%)	<0.0001
Preterm Birth	6 (7.06%)	1 (2.56%)	12(15.58%)	0.047

Table 4. Cytokine Correlation Matrix

	IL-6	IL-8	IL-10	IL-13	TNFa	ADI
IL-6	1.000	0.381	0.578	0.828	0.117	-0.155
p-value	--	<0.001*	<0.001*	<0.001*	0.099	0.029*
IL-8	0.381	1.000	0.129	0.304	0.176	0.013
p-value	<0.001*	--	0.068	<0.001*	0.013	0.853
IL-10	0.578	0.129	1.000	0.596	0.076	-0.099
p-value	<0.001*	0.067	--	<0.001*	0.282	0.160
IL-13	0.828	0.304	0.596	1.000	0.091	-0.098
p-value	<0.001*	<0.001*	<0.001*	--	0.198	0.167
TNFa	0.117	0.176	0.076	0.092	1.000	-0.003
p-value	0.099	0.013	0.282	0.198	--	0.966

Table 5. Median Cytokine Levels by Risk Group

	Low Risk (0-1 Risk Factors) N=85	Medium Risk (2 Risk Factors) N=39	High Risk (3+ Risk Factors) N=77	P-value
IL-6	10.04(6.62, 23.30)	8.86(6.20, 21.22)	10.56(6.53, 23.30)	0.909
IL-8	12.85(8.65, 30.49)	13.37(8.99, 36.16)	12.23(7.85, 32.29)	0.747
IL-10	35.99 (25.36, 59.08)	34.85(19.32, 51.08)	34.55(26.12, 48.57)	0.628
IL-13	7.84(5.60, 14.07)	7.63(5.32, 16.07)	7.99(5.30, 14.49)	0.871
TNF-a	8.13(6.08, 10.44)	7.73(6.02, 10.81)	8.06(6.72, 10.81)	0.632

Table 6. Median Cytokine Levels Stratified by Race

	Black Women Median(p25,p75)	White Women Median(p25,p75)	p-value
IL-6	8.80(5.28, 25.48)	10.56(7.51,23.30)	0.037*
IL-8	13.57(8.04, 40.23)	12.23(8.00, 24.62)	0.429
IL-10	35.99(25.23, 47.71)	34.85(23.84, 58.04)	0.393
IL-13	7.74(5.15, 15.46)	8.29(5.60, 14.49)	0.349
TNF-a	7.73(6.12, 10.44)	8.45(6.35, 10.76)	0.390

Table 7. Median Cytokine Levels Stratified by Risk and Race

	Low Risk	Moderate Risk	High Risk	p-value ¹
IL-6				
White	10.30(7.60, 20.47)	8.16(6.36, 17.71)	13.86(8.59, 26.44)	0.404
Black	8.87(5.65, 25.44)	8.91(5.82, 26.89)	7.85(4.18, 19.80)	0.895
<i>p-value</i> ²	p=0.237	p=0.792	p=0.054*	
IL-8				
White	10.17(7.49, 29.95)	12.28(8.95, 46.35)	12.72(8.60, 20.52)	0.452
Black	15.15(10.84, 30.49)	13.57(10.52, 28.42)	9.65(6.96, 59.44)	0.433
<i>p-value</i> ²	p=0.067	p=0.713	p=0.436	
IL-10				
White	42.31(28.94, 67.60)	28.40(16.34, 58.04)	32.07(22.07, 49.39)	0.114
Black	31.47(22.77, 47.00)	37.65(20.82, 45.21)	38.42(26.88, 48.57)	0.293
<i>p-value</i> ²	p=0.018*	p=0.571	p=0.330	
IL-13				
White	8.48(5.80, 14.01)	7.40(5.20, 13.48)	8.29(6.62, 14.49)	0.676
Black	7.40(5.00, 14.07)	7.82(5.65, 16.07)	7.87(4.76, 15.82)	0.982
<i>p-value</i> ²	p=0.347	p=0.832	p=0.511	
TNFα				
White	8.47(6.45, 11.07)	8.45(6.02, 10.30)	8.39(6.72, 10.44)	0.957
Black	7.73(5.94, 8.97)	7.40(6.26, 10.81)	7.83(6.72, 11.31)	0.339
<i>p-value</i> ²	p=0.191	p=0.777	p=0.984	

¹ p-value associated with testing for differences in median cytokine levels between risk groups within one race(Black or White).

² p-value associated with testing for differences between races within one risk group

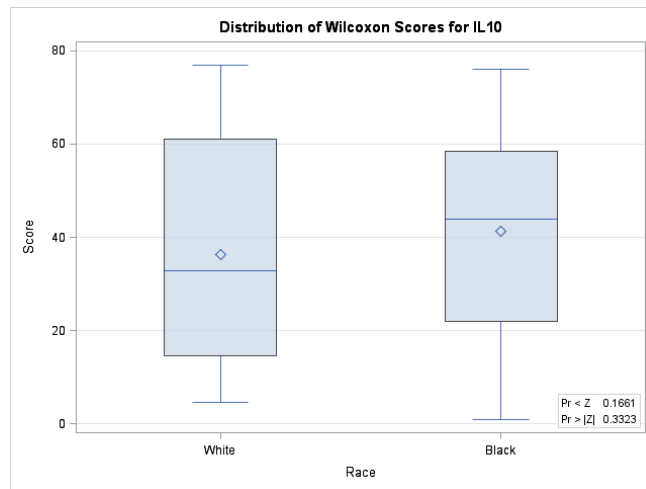
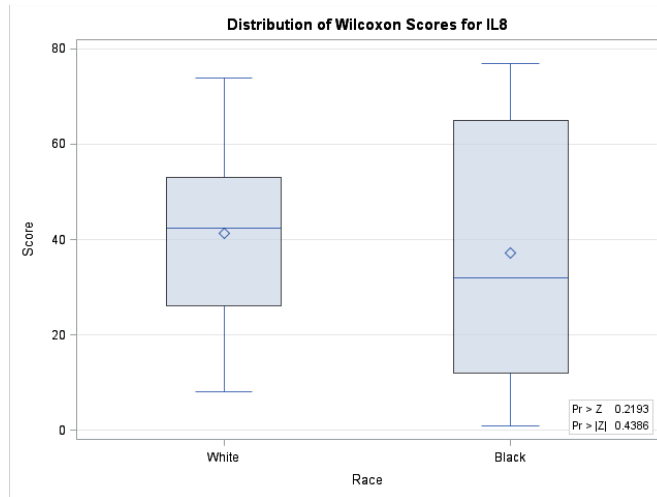
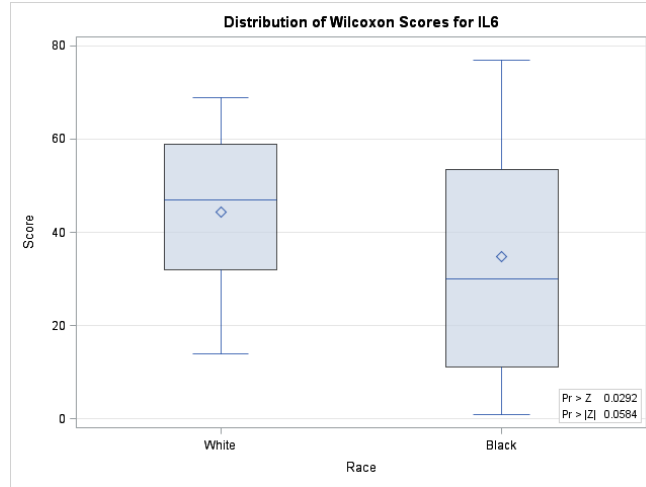


Figure 7. Racial Differences in Median Cytokine Levels in High Risk Group

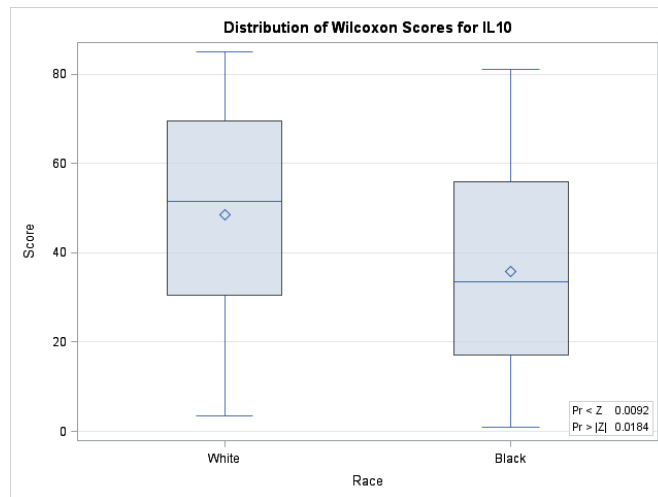
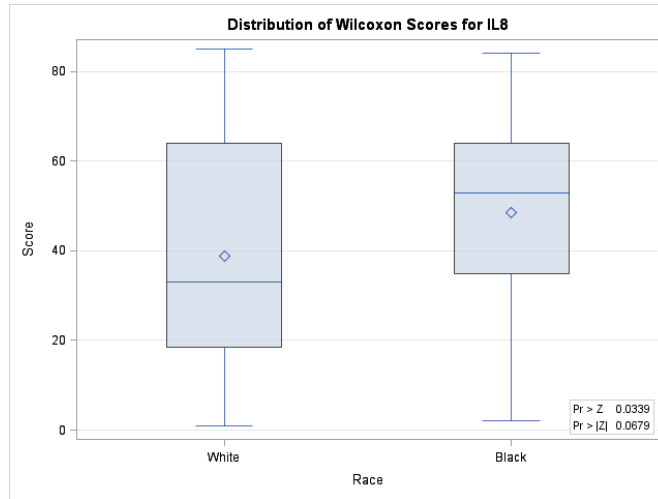
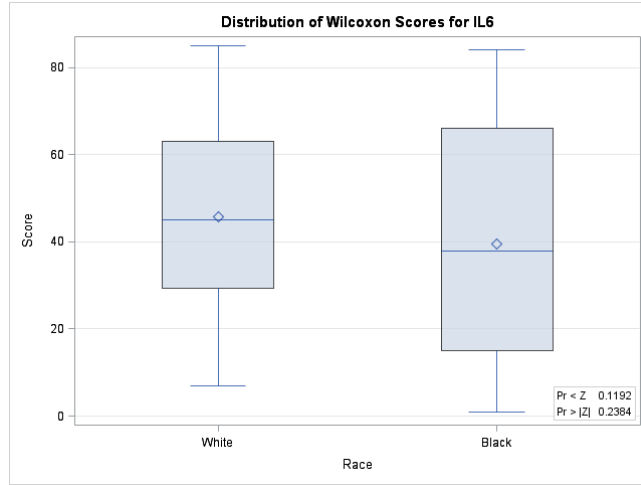


Figure 8. Racial Differences in Median Cytokine Levels in Low Risk Group

Table 8. Log Binomial Regression: Relative Risk of Being in Highest Quartile for Each Cytokine

	High IL-6 RR, 95%CI	High IL-8 RR, 95%CI	High IL-10 RR, 95%CI	High IL-13 RR, 95%CI	High TNFa RR, 95%CI
Risk Group					
Low	Ref	Ref	Ref	Ref	Ref
Medium	0.90(0.46, 1.78)	1.03(0.53, 2.02)	0.99(0.67, 1.48)	1.05(0.55, 2.04)	1.01(0.52, 1.95)
High	0.99(0.59, 1.69)	1.13(0.66, 1.92)	0.95(0.70, 1.31)	1.00(0.58, 1.73)	1.09(0.65, 1.86)
Age	0.99(0.95, 1.04)	0.99(0.95, 1.04)	1.01(0.98, 1.03)	1.01(0.97, 1.05)	0.96(0.92, 0.99)
Race					
White	Ref	Ref	Ref	Ref	Ref
Black	0.98(0.56, 1.71)	1.22(0.71, 2.10)	1.08(0.79, 1.48)	1.12(0.65, 1.92)	0.71(0.43, 1.19)

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