

PSYCHOSOCIAL STRESS AMONG PREGNANT WOMEN IN PUERTO RICO

by

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(Under the Direction of José F. Cordero)

ABSTRACT

Background: Preterm birth (gestational age less than 37 weeks), the leading cause of infant morbidity and mortality worldwide, disproportionally impacts pregnancies in Puerto Rico. Psychosocial stress during pregnancy has been associated with adverse pregnancy outcomes, including preterm birth, and has not been explored in Puerto Rico. Oxidative stress may represent one possible mechanism linking psychosocial stress to preterm birth. The purpose of this dissertation is to (1) describe psychosocial stress during pregnancy in Puerto Rico and to explore the relationships between psychosocial stress measures; (2) investigate oxidative stress as a potential physiologic response to psychosocial stress in pregnancy; and (3) investigate psychosocial stress as a potentially modifiable risk factor for preterm birth. **Methods:** Data from this dissertation came from the Puerto Rico Testsite for Exploring Contamination Threats (PROTECT) pregnancy cohort. Psychosocial stress was measured using the Perceived Stress Scale, Life Experience Survey, Center for Epidemiologic Studies-Depression scale, ENRICH Social Support Instrument, and two questions about neighborhood safety and quality adapted from the National Children's Study. Descriptive statistics and bivariate analyses were used to examine associations between demographic

characteristics and (1) psychosocial stress measures, (2) oxidative stress biomarkers and (3) preterm birth. Linear and logistic regression was used to examine associations between tertiles of psychosocial stress and oxidative stress biomarkers and preterm birth, respectively. **Results:** Approximately 10% of the PROTECT analytic sample delivered preterm. Perceived stress, negative life experiences, and neighborhood perceptions influenced depression through multiple pathways. No associations were observed between other indices of psychosocial stress and oxidative stress biomarker concentrations. High compared to low psychosocial stress was not associated with increased odds of preterm birth across any of the measures examined. **Conclusions:** Our study examined associations between multiple measures of stress among a pregnant Puerto Rican population. Findings from our study highlight the complex relationship between psychosocial stress measures. In the PROTECT pregnancy cohort, increased psychosocial stress was not associated with increased biomarkers of oxidative stress or increased odds of preterm birth.

INDEX WORDS: psychosocial stress, oxidative stress, preterm birth, pregnancy, social determinants of health, health disparities, Puerto Rico

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A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial
Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2019

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DEDICATION

Mom and Dad, this would not have been possible without you. Grandpa, the original Dr. Eick in the family, thank you for paving the way and always reminding me that there is a light at the end of the tunnel.

ACKNOWLEDGEMENTS

Mom and Dad, thank you for always believing in me and making this dream a reality. Thank you for your constant support of my academic pursuits, I could not have done this without you.

I would especially like to express my gratitude to my dissertation committee members: Drs. José Cordero, Kelly Ferguson, Andrea Swartzendruber, and Ye Shen. Thank you for the constant mentorship and guidance throughout this process. Dr. Cordero, I am eternally grateful for all the experiences you afforded me as a doctoral student, you opened more doors for me than I ever thought possible. Dr. Ferguson, you have been instrumental to me throughout my time as a doctoral student. Thank you for helping me grow as a researcher and an epidemiologist and for always being available for my endless amount of questions.

This would not have been possible without the support of my fellow MPH and PhD students. Thank you for listening to me, giving me feedback on ideas, reading many parts of this dissertation, and for the encouragement you have provided me throughout this process.

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CHAPTER 1

INTRODUCTION

Project Narrative

Psychosocial stress during pregnancy is linked to several adverse pregnancy outcomes of great public health concern, including preterm birth. Our study focuses on how psychosocial stress, measured through neighborhood perceptions, stressful life events, depression, perceived stress and social support, is associated with gestational age and explores oxidative stress as a possible mechanism linking stress to preterm birth. Results from our study may help to explain through what mechanisms psychosocial stress contributes to preterm birth.

Specific Aims

Preterm birth (PTB; gestational age <37 weeks), one of the leading cause of neonatal morbidity and mortality worldwide,¹ disproportionately impacts pregnancies in Puerto Rico.² The rates of PTB in Puerto Rico are some of the highest both in the U.S. and globally,³ with rates as high as 19.9% in 2006.⁴ The reasons for this high PTB rate are not well known. Maternal factors such as prenatal care, maternal age, or maternal education may not explain the observed increase. Although the rate of PTB on the island has since decreased to 11.5% in 2017,⁵ the PTB rate in Puerto Rico remains high relative to the rest of the U.S.⁵

Although there is a paucity of evidence regarding the effects lower socioeconomic status (SES) on Puerto Rican islanders, mainland U.S. Puerto Ricans

experience increased poverty, have higher levels of comorbid medical conditions, and higher PTB rates relative to other Hispanic subgroups and non-Hispanic whites.⁶ Stress disproportionately affects individuals of lower SES.⁷ Therefore, psychosocial stress may represent one possible mechanism contributing to Puerto Rico's high PTB rates.

It is hypothesized that psychosocial stress contributes to PTB through activation of the hypothalamic-pituitary-adrenal (HPA) axis, which increases cortisol production.⁸ Another possible pathway is through oxidative stress, which has been previously associated with psychosocial stress⁹⁻¹⁴ and is increased in mothers who go on to deliver preterm.^{15,16}

Some measures of psychosocial stress, such as perceived stress, depression, anxiety, and stressful life events have been associated with PTB, although results are inconsistent and population-specific.¹⁷ The neighborhood context may also be a substantial source of psychosocial stress and have been associated with symptoms of depression, increased perceived stress, lower social support,¹⁸ and increased risk of PTB.¹⁹ Social support, a buffer of psychosocial stress, may be indirectly protective against adverse pregnancy outcomes²⁰ and may contribute to resiliency.²¹

The relationship between different parameterizations of psychosocial stress measures has not been studied in Puerto Rico. However, literature has suggested that some measures such as social support and life events,²² neighborhood perceptions, and perceived stress and depression²³ are associated in African American populations. To expand on the literature, additional studies in different populations with prospective data collection on multiple psychosocial stress domains and a variety of potential confounders is needed.

Few studies have examined the relationship between psychosocial stress and birth outcomes among Puerto Ricans, although one prior study of predominately Puerto Rican women residing in the continental U.S. found that mid-pregnancy perceived stress increased the risk for PTB and low birth weight.²⁴ Given that many of the associations between stress and pregnancy outcomes are population specific, it is important to investigate these associations among Puerto Ricans islanders.

The mechanisms, magnitude, and associations among psychosocial stress factors and between these factors PTB in Puerto Rico islanders are unknown. Our primary goal for this dissertation is to examine the interrelationships of psychosocial stress measures, their associations with gestational age, and explore one possible mechanism through which this occurs. To accomplish this goal, we will use data from the Puerto Rico Testsite for Exploring Contamination Threats (PROTECT), an ongoing prospective cohort study designed to examine environmental risk factors for PTB in the Northern Karst region of Puerto Rico. We will accomplish the following specific aims:

Aim 1: Examine relationships between psychosocial stress measures among pregnant women in Puerto Rico.

Aim 2: Examine oxidative stress as a potential physiologic response to psychosocial stress in pregnancy.

Aim 3: Examine psychosocial stress as a risk factor for preterm birth.

CHAPTER 2

LITERATURE REVIEW

Overview of Preterm Birth

Preterm birth, defined as a birth <37 weeks gestation,²⁵ is one of the leading cause of neonatal morbidity and mortality worldwide.²⁶ Each year, roughly 15 million babies are born preterm globally.²⁷ An estimated 35% of neonates worldwide who die during the first month of life die due to causes directly related to preterm birth.²⁵ During 2017, 9.9% of infants born in the U.S. were preterm. Among U.S. states and territories, West Virginia, Mississippi, Alabama, and Louisiana all received “F” ratings by March of Dimes for having preterm birth rates above 11.5%. Along with these states, Puerto Rico and most states in the southeastern U.S. received “D” ratings for their 2017 preterm birth rates.⁵

Preterm births are categorized as being spontaneous or medically indicated and can be divided into subgroups based on gestational age: extremely preterm (<28 weeks), very preterm (28-32 weeks), moderately preterm (32-34.5 weeks) and late preterm (34.5-<37 weeks).²⁵ Early term delivery, which is not included in the preterm birth definition, includes births occurring between 37 and 38 weeks and 6 days gestation.²⁸

Pathways to Preterm Birth

Preterm birth is a multifaceted outcome and the underlying events that can lead to spontaneous preterm birth include preterm premature rupture of the membranes

(PPROM) but most of the underlying causes remained unknown.²⁵ Commonly accepted biological pathways leading to spontaneous preterm birth include: maternal fetal hypothalamic–pituitary–adrenal (HPA) axis activation, infection and inflammation, decidual hemorrhage, and pathologic over distension.²⁵

Parturition, or spontaneous labor, is divided four stages: (0) quiescence, (1) activation, (2) stimulation, and (3) involution.²⁵ The first stage, quiescence takes up 95% of the time during human pregnancy. During this stage, there are rarely contractions, which is primarily due to the lack of gap junctions in the pregnant myometrium.²⁵ When labor begins, there is an increase in the number of gap junctions. During activation, there is increased expression in the protein of myometrial gap junctions.²⁵ Uterine stretch occurs, resulting from fetal growth or activation of the fetal HPA axis.²⁵ This causes a biological cascade of events which leads to common pathway of spontaneous labor, where we see uterine contractions, cervical ripening, and fetal membrane activation.²⁵ This biological cascade is the 2nd stage, stimulation. The final stage, involution, involves placental separation and uterine contraction and ends with birth.²⁵ Both spontaneous preterm and full term births share this common pathway of partition. Spontaneous preterm labor is the result of external stimuli that cause the partition pathway to activate.²⁵

Premature rupture of the membranes (PROM) is the rupture of the membranes prior the beginning of labor and can occur in both preterm and full term birth.²⁹ Preterm PROM (PPROM) occurs when membranes rupture preterm and not in the context of preterm labor.³⁰ During pregnancy, the fetal membranes serve as a barrier preventing infection.²⁹ Fetal membranes rest on collagen, which provides the structural strength for

the membranes.²⁵ When the membranes have ruptured, the mother and her fetus are at increased risk of infection and other complications.²⁹

Medically indicated, or non-spontaneous preterm birth, is the final pathway leading to preterm birth.²⁵ Medically indicated preterm birth occurs when labor is initiated by medical intervention because of pregnancy complications that can affect the life and well-being of the mother and/or the fetus.²⁵ Well established medical indications for early elective delivery include preeclampsia, uncontrolled hypertension and others.²⁵

Causes and Risk Factors for Preterm Birth

An abundance of research has focused on understanding the biologic causes, medical conditions, and epidemiologic risk factors that increase the risk of preterm birth. Despite this, known risk factors only explain approximately 1/3 of the variation in preterm birth.³¹

Biologic factors that put women are at high risk for preterm birth include prior preterm birth, prior preterm labor, prior PPROM, cervical insufficiency, uterine overdistention, or having reproductive organ abnormalities such as short cervix.^{25,32}

Medical conditions that increase the risk for preterm birth include urinary tract and sexually transmitted infections, vaginal infections or bleeding, inflammation of the fetal membranes, and developmental abnormalities of the fetus.^{25,32} Maternal weight, specifically pre-pregnancy underweight or obese body mass index and low or excessive gestational weight gain, also place a woman at increased risk for delivering preterm.^{25,32} Pregnancy specific medical conditions include gestational diabetes mellitus, preeclampsia and eclampsia, uteroplacental thrombosis, fetal abruption, placenta previa, and rupture of the uterus.^{25,32}

Epidemiologic risk factors for preterm birth include low educational attainment, maternal age, being unmarried, and lifestyle factors such as smoking, alcohol consumption, and illicit drug use, as well as psychosocial stress, and unintended pregnancy.²⁵ Women less than 18 are at increased risk for delivering preterm,²⁵ whereas women older than 35 are also at increased risk because they are more likely to have other medical conditions, including hypertension and diabetes that may cause complications that require a preterm birth.³² The risk of preterm birth also varies by race. African-Americans have the highest rates of preterm birth in the U.S., followed by American Indian and Alaskan Natives, Hispanics, Asian and Pacific Islanders, and non-Hispanic whites.³³ Among Hispanic subgroups, Puerto Ricans have the highest rates of preterm birth.³³ Stress is hypothesized as one reason for these racial disparities.²⁵ Environmental contaminants, including phthalate exposure, also increase the risk for preterm birth.³⁴

Health Conditions Related to Preterm Birth

Preterm infants are predisposed to numerous health conditions at birth that can lead to significant life-long disabilities.²⁵ Complications from preterm birth arise from underdeveloped organ systems and include immature regulatory systems, respiratory distress syndrome, and neurodevelopmental problems.²⁵ Infants born preterm also have difficulty digesting food and are more likely to develop long term gastrointestinal problems.²⁵ Relative to infants born at term, preterm infants are more prone to infections, including pneumonia or sepsis and more than 65% of infants born premature have at least one infection during hospitalization.²⁵ After the initial discharge, late preterm infants also have a high rate of hospital admission, mostly due to infection.³⁵

Lastly, cardiovascular problems, hearing loss, and visual abnormalities are commonly seen among those born preterm.

Additional Adverse Perinatal Outcomes

The leading causes of infant mortality in the U.S. are preterm birth, birth defects, and sudden infant death syndrome (SIDS).¹ Birth defects, which range in severity, are structural abnormalities that can affect how almost any part of the body looks, works, or both.³⁶ Compared to full term, infants born preterm are twice as likely to have a major birth defect.³⁷ Different from birth defects is SIDS, which is a type of sudden unexplained infant death and often occurs during sleep or near the baby's sleep area.³⁸

Perinatal outcomes regarding birth weight include low birth weight and small and large for gestational age births. Low birth weight is a term used to describe babies who weigh less than 2500 grams at birth.³⁹ Two common reasons for low birth weight are preterm birth, because the baby has not had the time to gain weight in utero,³⁹ and fetal growth restriction. Infants are considered small and large for gestational age if they are weight less than the 10th and 90th percentile for gestational age, respectively.²⁵ Some preterm birth infants may be either small or large for gestational age.⁴⁰

Stress

Definitions and Domains of Stress

Hans Selye first defined stress in 1936 as the body's non-specific response to any demand.⁴¹ Today, stress is defined as the mental or emotional strain resulting from an adverse circumstance⁴² and the effects of anything that seriously harm homeostasis.⁴³ Stress can play a role in both the development and exacerbation of disease,⁴⁴ as every system in the body is influenced to some extent by stress. Physical

conditions associated with stress include rheumatoid arthritis, coronary heart disease, cancer, inflammatory bowel disease, and stomach ulcers.⁴⁵ Stress can also influence our mental health and has been linked to psychiatric diseases such as psychosis and schizophrenia.⁴⁵

Psychosocial stress is a type of stress that occurs due to social situations. Psychosocial stress may play a contributing role in adverse pregnancy outcomes, as it has been implicated as a risk factor and effect modifier for outcomes such as low birth weight, preterm birth, birth defects, and postpartum depression.^{17,46,47}

Psychosocial stress can be measured through biomarkers of stress and stress questionnaires that elicit a stress scale. Biomarkers are quantifiable characteristic of the biologic process and serve as objective indicators⁴⁸ whereas stress scales are more subjective indicators. These two measures do not always correlate.⁴⁸ Two of the most commonly studied biomarkers of stress are cortisol and oxidative stress. Cortisol is a biomarker of the HPA axis and provides a measure of acute or chronic stress levels over a given time period.⁴⁹ Oxidative stress is related to the immune system, where the production of reaction oxygen species (ROS) produces more inflammatory cells.⁴⁹

Stress scales measure domains of psychosocial stress. These domains include: external stressors, buffers and enhancers of stress, and perceived stressors.²² External stressors are major life changes or stressful life events.²² Differently, social support is a buffer of stress, this is one domain of psychosocial stress that may help foster resilience and may be protective against adverse pregnancy outcomes.²⁰ Enhancers of stress include perceived stressors, depression, and anxiety.²² Lastly, perceived stress incorporates the perception and impact of major life events, neighborhood perceptions,

and discrimination.²² Psychosocial stress is a combination of these domains. Thus, measuring one domain of psychosocial stress may fail to provide a complete picture of one's psychosocial well-being.

Each domain can be measured through different stress scales and the psychometric properties for use during pregnancy are not well established.⁵⁰ However, there are pregnancy specific stress scales that are more reliable than general stress measures because they include specific references to pregnancy, childbirth, and parenting that improve accurate recall and reporting.⁵¹ The best instruments for use during pregnancy are summarized in Table 2.1. These measures were chosen based on reliability and validity.⁵⁰

Table 2.1. Best currently available measures for different domains of psychological stress.

Domain	Instrument
Enhancer of stress	State-Trait Anxiety Inventory, trait form
Enhancer of stress	Edinburgh Postnatal Depression Scale
Perceived stress	Perceived Stress Scale
Psychosocial stress	Abbreviated Scale for the Assessment of Psychosocial Status in Pregnancy
External stressors	Perinatal Life Events Scale

Psychosocial stress differentially impacts minority groups,^{25,52} making it difficult to measure psychosocial stress across cultures. Furthermore, minority groups are 1disproportionally affected by low SES compared to non-Hispanic whites.⁵² Low SES is associated with both increased stress levels and preterm birth, making it difficult to disentangle stress and race as individual risk factors.⁵² The most prevalent types of psychosocial stress also varies by race. For example, anxiety is more commonly seen among Hispanics, whereas depression and racial stressors are more common among blacks.²⁵

Stress domains themselves are not causes of adverse pregnancy outcomes. Rather it is one of many epidemiologic risk factors and may affect the biologic processes, measured by biomarkers that are on the causal pathway to adverse pregnancy outcomes.

Biologic Pathways of Stress

One leading hypothesis is that stress leads to preterm birth through activation of the HPA axis. Stress may also lead to preterm birth through increased oxidative stress.

Cortisol

During acute episodes of stress, stress hormones are produced by the HPA axis and sympathetic nervous system (SNS) for the body's immediate use.⁴³ The SNS stimulates the adrenal medulla, which produces catecholamines.⁴³ At the same time, the hypothalamus produces corticotrophin releasing hormone, which stimulates the pituitary gland.⁴³ In response to stimulation, the pituitary produces adrenocorticotropin, which then stimulates the adrenal medulla to secrete cortisol, a type of glucocorticoid.⁴³ Cortisol and catecholamines promote lipolysis, which is where the body breaks down fats into usable energy sources.⁴³ During periods of acute stress, glucocorticoids suppress inflammation.⁵³

Chronic stress can cause the acute stress response to become maladaptive⁴³ and not suppress inflammation.⁵⁴ During periods of chronic stress, cortisol suppresses proinflammatory cytokine production, which are produced by immune cells.⁴³ The immune cells become unaffected to the increase in cortisol and cortisol is unable to stop inflammation.⁴³ Under these circumstances, stress will promote inflammatory cytokine production indefinitely.⁴³

During periods of high stress, the adrenal cortex increases maternal cortisol after activation of the HPA axis.⁵⁵ The increase in maternal cortisol can cross the placenta and increase fetal cortisol.⁵⁵ Transfer of maternal cortisol to the fetus also increases fetal corticotrophin releasing hormone (CRH), which is associated with preterm birth, decreased fetal growth, activation of the fetal HPA axis, and brain impairment.⁵⁵

Cortisol can be readily measured in urine, saliva, blood and hair, making it a useful biomarker of the HPA axis. Cortisol is highest in the morning and gradually decreases throughout the day and is lowest at night time.⁵⁶ Acute stress activates the HPA axis, thus increasing cortisol levels. Conversely, chronic stress inhibits the HPA axis. Therefore, people under chronic stress typically have lower baseline cortisol levels in the morning, flattening of the diurnal slope throughout the day, and higher cortisol levels at night. Cortisol concentration measured in the hair reflects the cortisol levels over several months. High stress individuals have on average, higher hair cortisol concentrations over time.

Cortisol and Psychosocial Stress

In non-pregnant populations, hair cortisol concentrations are highest among smokers, unemployed individuals, chronic pain patients, and those who have experienced stressful life events compared to reference groups.^{57,58} Compared to non-Hispanic whites, African Americans have higher salivary cortisol levels at the end of the day and have elevated hair cortisol concentrations and it is hypothesized that behavioral and psychosocial factors contribute to these racial differences.⁵⁶

Among pregnant women, those who subsequently develop post-partum depression have higher hair cortisol concentrations throughout pregnancy.⁵⁹ This finding

was confirmed with salivary cortisol levels, where low income pregnant women with lower daily salivary cortisol levels had an increased risk of developing post-partum depression.⁴⁷ Increased salivary cortisol has also been associated with increased perceived stress and anxiety among pregnant women.^{47,60}

Oxidative Stress

Oxidative stress is a biologic mechanism implicated within the stress response. Oxidative stress is the imbalance between the amount of reactive oxygen species (ROS) and the ability of the antioxidant system to neutralize them. The main cause of oxidative stress is ROS, which is produced by the mitochondria and NADPH oxidase (NOX) enzymes. Oxidative stress is measured through direct measurement of ROS, measuring of the damage to biomolecules, and via detection of antioxidant levels. ROS is highly unstable, therefore oxidative stress is often measured through indirectly through the biomarkers 8-isoprostane (8-iso) and 8-hydroxydeoxyguanosine (8-OH-dG), which are measures of lipid peroxidation⁶¹ and DNA damage,^{62,63} respectively. Compared to directly measuring ROS, 8-iso and 8-OH-dG provide a more accurate measure of oxidative stress because they are stable, readily detectable in bodily fluids and unaffected by lipids in the diet.⁶⁴

Oxidative Stress and Psychosocial Stress

It is hypothesized that oxidative stress affects the HPA axis and contributes to the stress response through: (1) altering RNA synthesis and stability, (2) altering the normal translocation of the glucocorticoid receptors from the cytoplasm to the nucleus, (3) increases in the stress-induced glutamate toxicity, and (4) modulating kinases and cysteine-rich, redox-sensitive proteins or the redox-dependent protein kinase C.¹²

After a challenge such as experiencing a major stressful life event, a prolonged change in the signaling network of the body, could lead to significant elevation in the production rate of damaging free radicals.⁶⁵ This elevated rate of free radical production would lead to prolonged and systemic oxidative stress as indicated by the increased accumulation of the oxidative stress biomarkers individuals experiencing stressful life events. For example, oxidative stress biomarkers are increased among abused women who experience stressful life events,⁶⁶ individuals who experienced a family death,⁶⁶ depression patients,⁶⁷ and those with poor self-blame and poor self-coping strategies.¹⁰ Animal models suggest that social isolation may be another source of increased oxidative stress.¹² Lastly, there is increased susceptibility to ROS among children whose parents were divorced¹² or adults who had a poor relationship with their parents during childhood.⁶⁸

Oxidative Stress During Pregnancy

Oxidative stress increases moderately throughout pregnancy and biomarkers of oxidative stress are significantly increased among women who go on to develop preeclampsia.¹⁶ Throughout the first trimester, increased oxidative stress can lead to early pregnancy loss, preterm birth, intrauterine growth restriction, and recurrent pregnancy loss.⁶⁹ During the second trimester, there is an increase in oxygen tension, increased ROS, and a burst of oxidative stress which can lead to the impairment of uterine perfusion and intrauterine growth restriction.⁶⁹ At the midpoint of pregnancy, 8-iso is significantly increased among women subsequently deliver preterm, whereas 8-OH-dG is higher among women who deliver low birth weight.⁷⁰ During the third

trimester, preterm birth, still birth, and intrauterine growth restriction can occur through damage to cellular components caused by oxidative stress.⁶⁹

Other Factors Contributing to Increased Oxidative Stress

Smoking is one of the most common behavioral factor that increases oxidative stress.⁷¹ Other endogenous factors that can increase oxidative stress are inflammation, cancer, ischemia, cell death,⁷² and traumatic brain injury.⁷³ Environmental exposures that have been associated with increased oxidative stress are phthalates,⁷⁴ phenols and parabens,⁷⁵ air pollution,⁷⁶ oil spills,⁷⁷ and pesticides.⁷⁸

Psychosocial Stress During Pregnancy

Psychosocial stress during pregnancy is common, a recent study of urban women found 78% of pregnant women experienced low or moderate stress during pregnancy.⁷⁹ Minority women are especially vulnerable to increased psychosocial stress due to inadequate social support, racism, and anxiety.^{17,80}

Compared to non-Hispanic whites, African-Americans are more likely to deliver preterm and low birth weight, with racism and chronic stress contributing to this racial disparity.^{17,81} Beyond racial differences, victims of domestic violence and those with depression have increased odds of experiencing psychosocial stress during pregnancy.⁷⁹ Women with depression and anxiety are also more likely to deliver low birth weight and have shorter gestation relative to those without.^{44,82} In addition to depression and anxiety, perceived stress increases the likelihood of delivering preterm.¹⁷ On the contrary, social support is inversely associated with birth weight.⁸¹ It is hypothesized that having good social support allows for women to adopt a healthier lifestyle, thereby reducing her stress levels.⁸¹

Interventions aimed at stress reduction, specifically yoga, physical relaxation and meditation, counseling, and improved access to resources may help decrease the preterm birth and low birth weight rates among high risk women.^{81,83-85} These interventions have demonstrated prior success.

Women who experience natural disasters during pregnancy are at increased risk for psychosocial stress and adverse birth outcomes. Among women who were pregnant or became pregnant immediately after Hurricane Katrina, those who experienced hurricane specific severe events were 16 times more likely to develop post-traumatic stress disorder⁸⁶ and were 50% more likely to have current perceived stress.⁸⁷ Women experiencing hurricane specific severe events also had an increased risk of preterm birth, low birth weight, and induction of labor.^{86,87} Some of these associations have persisted over time. For example, seven years after Hurricane Katrina, pregnant women who were worried about another hurricane had decreased gestational age of about 3.2 days.⁸⁸

The timing of the event is also important predictor of birth outcomes. Women who experienced 9/11 during the first trimester experienced a preterm birth much earlier in gestation than women who experienced 9/11 during subsequent trimesters.²⁵

There is no clear consensus on how psychosocial stress contributes to adverse pregnancy outcomes and another body of literature suggests that there is no association.¹⁷ Differences in results are often attributed to different stress definitions, methodological approaches, study populations, timing of scale administration, and use of different instruments or scales. Furthermore, most stress scales have no established

cut points for high and low stress, making it difficult to consistently define high and low stress.

Neighborhood Stress

Neighborhoods are clusters of people living close to one another within a defined geographical area.⁸⁹ The quality of one's neighborhood may be a significant source of stress¹⁹ and an important predictor of health disparities.⁹⁰ A low SES neighborhood can contribute to mental and physical health as a direct result of the built environment. Low SES neighborhoods may be disproportionately affected by a lack of infrastructure, green space, grocery stores, hospitals or clinics, child care, and/or public school systems.

Each of these factors could contribute to individual stress levels⁹¹ and residents of low SES neighborhoods have higher levels of psychosocial stress.⁹² Poor infrastructure may contribute to the unemployment rate and uninsured population, whereas relying on convenience stores for groceries may make it difficult for families to provide healthy food options. The lack of quality school systems may also lead to insufficient education attainment. Thus, these individuals may be more likely to engage in vandalism and drug use that contribute to the poor social environment.⁹³ Individuals who are SES disadvantaged are also more likely to experience stressful life events, such as violent crime.⁹⁴

African-Americans are disproportionately represented in low SES neighborhoods.⁹⁵ Other minority groups, such as Hispanic immigrants, may also be affected by SES disadvantage due to cultural or linguistic barriers, policies affecting their ability to work, and a lack of access to care.⁹⁶

Measures of Neighborhood Stress

There is no commonly accepted definition for neighborhood deprivation or neighborhood perceptions. Nonetheless, neighborhood deprivation is generally defined using objective census measures and includes measures of poverty, housing, occupation, employment, education, residential stability, and racial composition.⁹⁰

Neighborhood perceptions are measured using stress scales that contain questions that are specific to neighborhoods. These scales pertain to different aspects of one's neighborhood, such as neighborhood crime and safety, social cohesion, neighborhood problems, and neighborhood physical environment.^{19,66,97-99} Neighborhood deprivation and neighborhood problems may not always correlate. However, a previous study showed that that neighborhood deprivation was positively associated with perceived neighborhood problems,¹⁰⁰ indicating that some aspects may overlap.

Neighborhood and Gestational Age

Compared to women in the most advantaged areas, women in the most deprived areas have increased odds of preterm birth and low birth weight. Similarly, the rates of preterm birth, still birth, and neonatal death are highest among women who live in the lowest income and education quartiles.¹⁰¹ Women who experience stressful life events during pregnancy are also more likely to deliver preterm and low birth weight with neighborhood deprivation modifying this relationship.^{102,103}

Within strata of race, non-Hispanic white and non-Hispanic black women in the most disadvantaged have higher odds of preterm birth and low birth weight compared to most advantaged.⁹² When adjusting for race, these associations in some studies do not persist.⁹² It is possible that race is a surrogate for other factors explaining these

associations. Thus, adjusting for race may be fully accounting for the unmeasured confounding in some scenarios.⁹²

Neighborhood and Psychosocial Stress

Neighborhood perceptions are a significant source of psychosocial stress. Negative neighborhood perceptions have been associated with symptoms of depression, increased perceived stress, and lower social support.¹⁸ Perceived residential detrition, which includes concerns over safety, declines in home values, and detrition of commercial structures, is also positively correlated with stress.⁹⁸ Among pregnant women, negative neighborhood perceptions has been associated with depression, with social support mediating this relationship.^{18,104}

On the contrary, those who have favorable attitudes towards their neighborhoods have decreased levels of perceived stress¹⁰⁵ and children in higher SES neighborhoods are more likely to report better quality of life.¹⁰⁶

Neighborhood and Biomarkers of Stress

Residents of disadvantaged and low income neighborhoods are more likely to have higher salivary cortisol levels¹⁰⁷ and shortened telomere length,^{66,108,109} a biomarker of stress. The associations between neighborhood perceptions and telomere length remained consistent after adjusting for psychosocial stress.⁹⁷ Psychosocial stress was also an independent predictor of shortened telomere length.⁹⁷

Social Ecological Model Adapted to this Dissertation

The social ecological model of health behavior is the overarching model that can best encompass the goals of this dissertation. We have developed our own social ecological model to explain how events and interactions at each level of our daily life

can influence our behaviors and ultimately our health. This type of model was chosen because it takes into consideration multiple levels that cannot only influence each other, but ultimately impact the health behaviors of individuals.¹¹⁰ This model considers the dynamics of our environment, policies, communities (cultural, neighborhood, family, workplace, etc.), and personal, social, and psychosocial states.¹¹⁰ For the purposes of this model, the multiple levels of influence which we have chosen to include are summarized in Table 2.2 below and are presented visually in Figure 2.1.

Table 2.2. Levels of influence within social ecological model

Levels	Application
Intrapersonal	-Demographics -Biology -Psychosocial -Personal experiences
Interpersonal (Perceived Environment)	-Social network -Perceived crime and safety -Family Situation -Attractiveness of neighborhood -Comfort at home -Social support (friends or family)
Community	-Social climate -Religious/place of worship -Sports leagues -Public recreation areas (parks, gym)
Physical environment	-Crime rate -Built environment -Abandoned buildings -Demographics of community -Ability to walk places
Policy	-Health care policies -Park policies -Home prices -Parking regulations -Land use -Public school system

At the individual level, demographics and individual biology can work to shape and influence personal experiences. For example, someone who is African-American

may be more likely to experience racism, a type of psychosocial stress. This in turn could lead to increased cortisol levels. If this same person is a single parent or has a negative home life, then the family situation may also be contributing to increased stress levels. The interpersonal level is what makes up our perceived environment. This considers how we would rate the built environment around us, including the attractiveness of our neighborhood and how safe or unsafe we might feel in our own home or walking down the street. Interpersonal factors also include our social network, both family and friends. A strong social network is generally associated with better social support, and in turn may help to counter some of the adverse effects of stress. Relating this back to the intrapersonal level, a negative home life would contribute to feelings of low social support and thus increasing biomarkers of stress.

Community structures affect social and interpersonal interactions and events that can either foster or inhibit positive social culture. If there is easy access to churches or places of worship, recreation centers, sports leagues, or other areas that support social gatherings, this might encourage neighborhood residents to make friends or spend more time outside, which could foster a large social network. A large social network could be a source of social support, which could lead to better health outcomes since social support is thought to be a source of resilience.

The physical environment level refers to the built environment of the neighborhood where individuals live. This encompasses neighborhood crime rate, number of abandoned buildings, community demographics, and walkability. These factors are all correlated and can be positive or negative depending on each community. If there is a high crime rate, few sidewalks, and many abandoned buildings,

a resident may feel unsafe walking places, which may lead to them feeling unsafe within their neighborhoods. Feeling unsafe in the neighborhood could cause someone to stay inside their personal home more often, leaving them with limited time and opportunity to foster relationships with others in the community.

A low SES neighborhood without sidewalks or green space may deter someone from exercising. Residents of low SES neighborhoods often will have corner or convenient stores instead of grocery stores, which inhibit their ability to eat healthy or feed their families healthy meals. Cramped living conditions, such as a small apartment with a large family living in it, may also be a contributing factor to increased stress levels.

Policy is the broadest, overarching level, which incorporates rules and regulations that help shape all the levels within the model. Limited access to public and private schools in the neighborhood could deter children from getting the education they need to go on to college or get a job. This can have a negative effect on the social climate of the neighborhood if children aren't encouraged in school to learn, grow, and thrive in society. Someone who is uneducated may be more likely to partake in destructive behaviors such as stealing, vandalism, and drug and/or alcohol abuse. Each of these poor outcomes could contribute to high rates of crime in a neighborhood. Inadequate land use, home prices, parking regulations, and unavailability of green space could contribute to the decreased walkability of the neighborhood, therefore inhibiting exercise and perceived neighborhood comfort or safety.

The policy level could inhibit access to care if there are no clinics or hospitals within certain demographic areas. Limited access to clinics could prevent a woman from

entering prenatal care on time or could prevent her from getting prenatal vitamins. If clinics or hospitals are easily accessible, residents of low SES neighborhoods may not have insurance or may not have adequate insurance to see a health care provider.

To illustrate the effects of policy on individual health on a broader scale, the states that have not expanded Medicaid had the highest uninsured and poverty rates in 2015.¹¹¹ In Texas and Florida, there are 3 million residents who do not have health care as a direct result of not expanding Medicaid.¹¹¹ This has detrimental implications for the health and well-being for these individuals. Cost is the overwhelming barrier for individual to not seek health care.¹¹¹ Cost has a direct implication to prenatal care. Among urban women who had not received prenatal care, over half cited cost as the main reason for not doing so.¹¹² Similar findings have been observed in the rural setting, where 87% of mothers not receiving prenatal care said they could not afford it.¹¹² Adults who are well off are still more likely to underuse health care when they have to pay anything out of pocket.¹¹¹ This could mean that the rising cost in co-pays or medications could lead individuals with and without insurance to not get the care they need. Approximately 9% of individuals in the U.S. are not proficient in English and more than half of adults in the U.S. cannot correctly define common terms in health care, such as premiums.¹¹¹ Not providing care in native languages and not fully understanding the costs are additional policy wide issues that have effects at the individual level.

An “onion” structure, outlined in Figure 2.1 below, is used as a visual representation of our social ecological model.

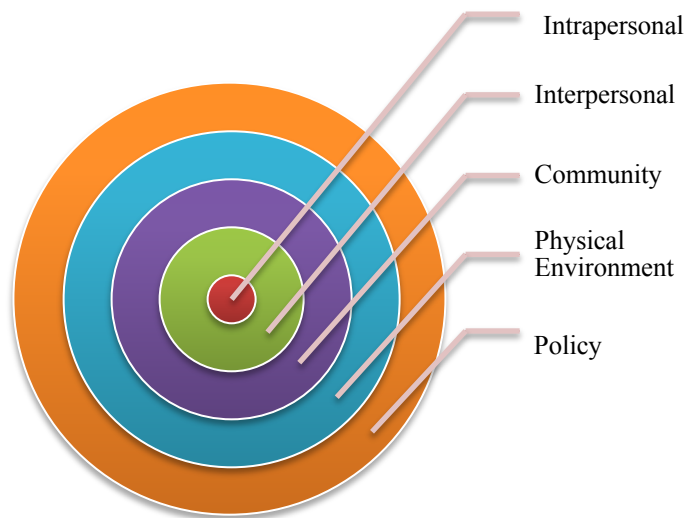


Figure 2.1. Social ecological model adapted to this dissertation.

CHAPTER 3

DISSERTATION METHODS

Study Population

Data for this dissertation comes from the Puerto Rico Testsite for Exploring Contamination Threats (PROTECT) cohort. PROTECT is a Superfund Research Center designed to examine the role of environmental contaminants in the etiology of preterm birth. PROTECT has developed an ongoing prospective cohort study of pregnant women in Puerto Rico who reside in the Northern Karst Region. The Northern Karst Region is home to most of Puerto Rico's superfund sites, where these sites overlie karst aquifers and can lead to water contamination. Water sampling of the aquifers in this region consistently show presence of pesticides, phthalates and other contaminants. Women included in this dissertation delivered between 2011 and 2017.

Women in PROTECT are recruited early in pregnancy up to 20 weeks gestation from 7 prenatal clinics and hospitals and are followed until delivery. Women are eligible for inclusion in PROTECT if they are between 18-40 years of age, reside in a municipality in the Northern Karst region, did not use oral contraceptives for at least three months prior to pregnancy, did not use *in vitro* fertilization to become pregnant, and were free of pregnancy complications. Women have 3 separate study visits, occurring between 16-20 weeks, 20-24 weeks, and 24-28 weeks. Participants provided demographic information via questionnaire at the 1st study visit. The study visits were timed with period of rapid fetal growth and routine clinical visits. During follow up

detailed data on medical, social, and environmental factors are collected as well as biological samples. All women provided informed consent and the Institutional Review Board at the University of Puerto Rico, University of Georgia, Northeastern University, and University of Michigan approved the PROTECT study.¹¹³

Women were included in the analytic sample for the first and third aim if they provided questionnaire information on any of the psychosocial stress measures and had gestational age at birth information available. The analytic sample for the second aim is a subset of women included in the first and third aim who additionally had urine samples measured for oxidative stress biomarkers.

Aim 1

Objective

Investigate relationships between psychosocial stress measures among pregnant women in Puerto Rico and to describe psychosocial stress in the PROTECT population.

Hypothesis

We hypothesized that some domains of psychosocial stress would be associated or correlated. Specifically, we hypothesized that we would observe correlations and/or associations between scores on the following scales based on our literature review:

- Neighborhood perceptions and perceived stress^{23,114}
- Neighborhood perceptions and depression¹⁰⁴
- Neighborhood perceptions and negative life experiences¹¹⁵
- Negative life experiences and depression¹¹⁶
- Social support and depression¹⁰⁴
- Neighborhood perceptions and social support¹⁰⁴

- Perceived stress and depression¹¹⁷

Methods

Psychosocial Stress Measures

Life Experiences

Women completed the Life Experiences Survey (LES) at the 2nd visit. The LES is a self-reported questionnaire of 38 life events where women are asked to rate if each event occurred (yes/no) within the last year.¹¹⁸ If the event occurred, women were asked to rate their perception of each event on a 7 point Likert scale with responses ranging from -3 (extremely negative) to +3 (extremely positive), with 0 being no impact. If women reported no life events, they were given a score of 0 to ensure that they were not dropped from analyses. Only events that were rated as being between -3 and -1 were included in our analysis because we were only interested in events that were perceived as negative. A summary measure of negative life events was created by summing up all the responses that were coded as -3, -2, and -1. If any question on the LES was missing, the overall score was coded as missing. This continuous measure was used in all analyses. High scores on the LES were consistent with increased negative perceptions of life events.

Depression

The Centers for Epidemiologic Studies-Depression (CES-D) was administered at the 3rd visit. The CES-D consists of 20 questions and is a screening test for depression which measures symptoms of depression according to the Diagnostic and Statistical Manual.¹¹⁹ Questions are designed to measure how often on a given day women experience depressive symptoms. Responses are on a 4 point Likert scale and range

from “rarely” (a score of 0) to “majority of the time” (a score of 3). Questions that were positively stated were reverse coded so that higher scores on each question indicated increased feelings of depression. If one question on the CES-D was missing then the overall score was missing. Responses to each question were summed to create a continuous measure of depression symptomology where higher scores were consistent with increased feelings of depression.

Social Support

The ENRICH Social Support Instrument (ESSI) is a 7-item scale which measures four types of social support: emotional and information social support, tangible social support, affectionate social support, and positive social interaction.¹²⁰ The ESSI was administered during the 3rd visit. Women were asked how often they feel they have social support, responses ranged from “rarely or none of the time” (a score of 1) to “all the time” (a score of 5). In PROTECT, data on marital status was collected at baseline and was not collected again during subsequent visits to eliminate redundancy. Thus, using a previously established method,¹²¹ we used baseline marital status (single (a score of 1) and married or living together (a score of 4)) in the ESSI scoring although it was not explicitly asked as part of this survey. As with the LES and CES-D, the overall score was coded as missing if one question was missing and responses were summed to create a continuous measure of social support. Higher scores were indicative of higher social support.

Neighborhood Perceptions

Neighborhood perceptions (NP) were based on responses to two questions adapted from the National Children’s Study.¹²² At the 2nd study visit, women are asked

to rate the quality of their neighborhood in response to the question “In your opinion, your neighborhood is...” Answer options are based on a 4 point Likert scale and range from “a good place to live” (a score of 1) to “not a very good place to live” (a score of 4). They were also asked about their perception of neighborhood safety using the question “You feel that your neighborhood is...” with answer options on a 4 point Likert scale ranging from “very safe” (a score of 1) to “very unsafe” (a score of 4). Responses to each question are summed to get an overall measure of neighborhood perception. If either question is missing then the overall measure is coded as missing. Higher scores on both questions and on the overall measure indicate unfavorable neighborhood perceptions.

Perceived Stress

The Perceived Stress Scale (PSS) was administered during the 3rd visit. The PSS is composed of 10 questions and is a current measure of stress and is designed to measure the extent to which one feels that situations in their life are stressful.¹²³ Responses to each question were ranked on a 5 point Likert scale. Responses ranged from “never” (a score of 0) to “almost always” (a score of 4). Positively stated items were reverse coded so that higher scores were always indicative of higher stress levels. Responses to each question were summed to create a continuous measure of perceived stress, where higher scores were associated with increased perceived stress. If any question on the PSS scale was missing then the overall PSS score was coded as missing.

Covariates

Body mass index (BMI; kg/m²), maternal age at delivery, maternal education,

employment status, marital status, alcohol use, smoking, and insurance status were included as potential covariates in our analyses. In model building, covariates that changed point estimates by greater than 10% were retained in our final models. These covariates are summarized in Table 3.1.

Table 3.1. Coding of covariates for Aim 1.

Covariate	Variable Type	Coding
Prepregnancy BMI (kg/m ²)	Categorical	<18.5 18.5-24.9 25-29.9 >30
Maternal age, years	Categorical	18-24 25-29 30-34 ≥35
Maternal education	Categorical	<High school High school or equivalent Some college or technical school ≥College degree
Employment status	Categorical	Unemployed Employed
Marital status	Categorical	Single Married Living together
Alcohol consumption	Categorical	Never Before pregnancy Currently drinking
Smoking status	Categorical	Never Ever Current
Insurance status	Categorical	Private Public Uninsured

Abbreviations: BMI, body mass index

Statistical Analysis

Descriptive statistics, including means, standard deviations (SDs), frequencies, and counts were used to examine maternal demographic characteristics and psychosocial stress measures. Unadjusted linear models were used to examine

differences between covariates and psychosocial stress measures. We examined the mean and SD of our five psychosocial stress measures across all demographic characteristics. Pearson's correlation coefficients were used to examine correlations between all psychosocial stress measures.

To test the associations listed in the hypothesis, we used linear regression models to calculate crude and adjusted beta coefficients and 95% confidence intervals (CI). Stress measures were included as both independent and dependent variables depending on the model. Models were developed based on our literature review and a final conceptual model was developed based on associations observed in the PROTECT study population. We also explored other associations between stress measures and effect measure modification (i.e., mediation and moderation). We examined models with stress measures in the same model to see how the stress measures work together.

P-values <0.05 were considered statistically significant. P-values for interaction were considered statistically significant at $p < 0.10$.

Sample Size Estimation

The minimum difference in LES, PSS, CES-D, ESSI, and NP we are able to detect in PROTECT was calculated using the sample size equation $N = ((Z_{\frac{\alpha}{2}} + Z_{\beta})^2 * 2 * \sigma^2) / d^2$. In this equation, N corresponds to total sample size for each psychosocial stress measure, $Z_{\frac{\alpha}{2}}$ and Z_{β} correspond to the z-score for α and β , respectively. Lastly, σ corresponds to the standard deviation of the psychosocial stress measure and d is the minimum difference in the psychosocial stress measure that we are able to detect. An α of 0.05 and power of 0.8 was used in each equation. Calculations were based on a

sample size of 861, 826, 795, 820, and 897 and σ of 4.22, 6.82, 9.02, 4.25, and 0.84 for each psychosocial stress measure, respectively. We are powered to detect a difference of 0.57 on the LES, 0.94 on the PSS, 1.27 on the CES-D, 0.59 on the ESSI, and 0.11 on the NP scales.

Limitations

Our assessment of psychosocial stress is limited in that no baseline measure of stress was available in PROTECT and that no objective measure of stress was included in our analysis. Second, the questionnaires related to stress were only asked once and generally stress questionnaires were administered at the same study visit. Thus, it is not possible to examine trends of stress throughout pregnancy. Repeating stress questionnaires and other stress measures would provide better evidence of how stress measures may predict one another. Lastly, we have limited generalizability to other populations and our study population included women from one region of Puerto Rico and the interrelationship between stress measures may differ in other populations.

Aim 2

Objective

Investigate oxidative stress, measured by oxidative stress biomarkers, as a potential physiologic response to psychosocial stress in pregnancy.

Hypothesis

We hypothesized that women with increased scores on the psychosocial stress questionnaires would have increased oxidative stress biomarkers after adjusting for individual level covariates.

Methods

Exposure

The main exposures for this aim, NP, LES, PSS, CES-D, and ESSI, have been previously described within the methods section for aim 1.

Outcome

The Eicosanoid Core Laboratory at Vanderbilt University Medical Center (Nashville, TN) measured free 8-isoprostane and its major metabolite using gas chromatography-negative ion chemical ionization-mass spectrometry in an additional 469 samples (N= 273 at visit 1, N= 338 at visit 2, N= 174 at visit 3).

Values below the limit of detection (LOD) were replaced by LOD divided by the square root of 2. For all urinary oxidative stress biomarkers, we adjusted for urine dilution with specific gravity (SpG). Oxidative stress biomarker concentrations were corrected for SpG using the formula: $Ox_c = Ox[(1.02 - 1)/(SpG - 1)]$, where 1.02 is the median SpG in the PROTECT population, Ox is the oxidative stress biomarker concentration as measured, and Ox_c is the SpG corrected oxidative stress measure.¹²⁴ We used the geometric average of oxidative stress biomarkers across pregnancy (visits 1-3) to obtain one averaged measure of oxidative stress across for each participant. This was done to ensure a more stable estimate of oxidative stress. All oxidative stress biomarkers were natural log transformed to ensure normality.

Covariates

Prepregnancy BMI (kg/m²), maternal age in years at delivery, maternal education, employment status, marital status, alcohol use, smoking, and insurance status were included as potential covariates in this analysis. The complete list of covariate coding is available in Table 3.1. During model building, covariates that

changed the point estimate of psychosocial stress measures by greater than 10% were retained in all final adjusted models.

Statistical Analysis

Frequencies, counts, means, and standard deviations (SDs) were used to describe the demographic characteristics of our study population. The distribution of oxidative stress biomarkers were examined using geometric means, geometric SDs, and selected percentiles. Linear models were used to obtain parameter estimates and standard errors between continuous and categorical covariates and oxidative stress biomarkers. P-values corresponding to these tests were used to determine differences between these covariates and oxidative stress biomarkers.

Linear regression models were used to determine crude and adjusted beta estimates and 95% confidence intervals (CI) for the associations between individual measures of psychosocial stress in tertiles and oxidative stress biomarkers. We checked standard linear regression assumptions by examining QQ-plots for each model. Beta estimates were converted to % difference in oxidative stress biomarkers for ease of interpretation.

P values <0.05 were considered statistically significant.

Sample Size Estimation

The minimum difference in free 8-isoprostane we are able to detect in PROTECT was calculated using the sample size equation $N = ((Z_{\frac{\alpha}{2}} + Z_{\beta})^2 * 2 * \sigma^2) / d^2$. Using a sample size of 438 for free 8-isoprostane, an α of 0.05, power of 0.8, σ of 0.429 for free 8-isoprostane, we are powered to detect a difference of 0.005 ng/mL and in free 8-isoprostane, respectively.

Limitations

This aim is not without limitations. First, although we examined many domains of psychosocial stress, there may be other domains of stress, such as anxiety, which may be important in this context and were not explored in this analysis. Additionally, we have limited generalizability to Puerto Ricans in other parts of the island and in the mainland U.S., as well as other Hispanic populations.

Aim 3

Objective

Investigate psychosocial stress as a potentially modifiable risk factor for preterm birth.

Hypothesis

We hypothesized that women with higher scores on the psychosocial stress questionnaires would have decreased gestational age and be more likely to deliver preterm.

Methods

Exposure

The main exposures for this aim, NP, LES, PSS, CES-D, and ESSI have been previously described within the methods section for aim 1.

Outcome

Our outcomes of interest are gestational age and preterm birth. Gestational age was measured by a combination of last menstrual period (LMP) and best obstetrical estimate of gestational age obtained at the first ultrasound. Preterm birth was defined as delivery occurring prior to 37 weeks gestation.

Covariates

Covariates included in aim 3 were the same as those included in aims 1 and 2 and include prepregnancy BMI (kg/m^2), maternal age in years at delivery, maternal education, employment status, marital status, alcohol use, smoking, and insurance status. A detailed description of all included covariates is available in Table 3.1 within the methods section of aim 1. Covariates that changed the point estimate of any psychosocial stress measure by greater than 10% were retained in all final adjusted models.

Statistical Analysis

Frequencies, counts, means, and SDs were used to describe the demographic characteristics of our study population. Chi-squared tests were used to determine differences between our categorical demographic characteristics and preterm births.

Linear regression models were used to determine crude and adjusted beta estimates and 95% CIs for the associations between all psychosocial stress measures in tertiles and gestational age using separate models for each stress measure. Standard linear regression assumptions were checked by examining QQ-plots for each model. We examined associations between all stress measures and preterm birth by calculating crude and adjusted odds ratios and 95% CIs using logistic regression models. We additionally assessed for interaction between psychosocial stress measures based on constructs developed in aim 1.

Statistical significance was assessed at $p\text{-value} < 0.05$.

Sample Size Estimation

The minimum difference in gestational age that we are able to detect was calculated using the sample size equation, $N = ((Z_{\frac{\alpha}{2}} + Z_{\beta})^2 * 2 * \sigma^2) / d^2$. Using a sample size of 1,179, an α of 0.05, power of 0.8, and σ of 2.53, we are powered to detect a difference of 0.12 weeks gestation.

Limitations

Our study is not without limitations. First, it is difficult to quantify psychosocial stress. However, we included five different measures of psychosocial stress in this analysis, each of which has been used in other populations. An additional limitation is the timing of our psychosocial stress measures, which were administered at the 2nd and 3rd study visits. There were no baseline or repeat measures of psychosocial stress available in PROTECT. A prior study among Puerto Ricans in the mainland U.S. has shown that perceived stress at mid-pregnancy (mean 21.3 weeks gestation), but not early pregnancy (mean 12.4 weeks gestation), is associated with increased odds of preterm birth.²⁴ In our study population, most psychosocial stress measures were administered at later in pregnancy (mean 23.8 weeks gestational and 28.1 weeks gestation for the second and third study visit, respectively). Lastly, our study sample is a subset of pregnant women in the Northern Karst region of Puerto Rico, thus our results may not be generalizable to all women in Puerto Rico or other Hispanic subgroups.

CHAPTER 4

PSYCHOSOCIAL STRESS AMONG PREGNANT WOMEN IN PUERTO RICO: A PATH ANALYSIS

Background: Psychosocial stress during pregnancy has been associated with adverse pregnancy outcomes including preterm birth. Living in a deprived neighborhood is associated with higher levels of depression, perceived stress, increased stressful life events, and ultimately preterm birth. However these associations have not been explored in Puerto Rico, an area with high preterm birth rates. Our objective is to develop a conceptual model describing the interrelationships between measures of psychosocial stress among pregnant women in Puerto Rico.

Methods: We used data from the Puerto Rico Testsite for Exploring Contamination Threats cohort (N=1,047) to examine associations between different domains of psychosocial stress using path analyses. Psychosocial stress during pregnancy was assessed using validated measures of perceived stress, negative life experiences, neighborhood perceptions, depression, and social support at the 2nd and 3rd study visits (median 23.4 and 27.1 weeks gestation, respectively).

Results: Perceived stress, negative life experiences, and neighborhood perceptions influenced depression through multiple pathways. Our model indicates that perceived stress had the strongest direct effect on depression ($\beta=0.57$, standard error (SE)=0.04), i.e. one standard deviation (SD) increase in perceived stress was associated with a 57% SD increase in depression. Neighborhood perceptions directly influenced negative

life experiences ($\beta=0.08$, $SE=0.15$) and perceived stress ($\beta=0.12$, $SE=0.25$) and was indirectly associated with depression ($\beta=0.10$, $SE=0.08$).

Conclusions: Our study examined associations between multiple measures of psychosocial stress among a pregnant Puerto Rican population. Results from our study indicate that the neighborhood context influences depression through multiple pathways. Our findings highlight the complex relationship between psychosocial stress measures. Future research utilizing these measures should consider the mediating and moderating pathways we identified when examining diverse parameters of stress to adverse health outcomes.

Introduction

Psychosocial stress stems from perceptions of threats which result in discomfort, emotional tension, and difficulty adjusting.¹²⁵ Stress can be triggered from many different sources, including anxiety and extreme stressful life events, such as a family death and job loss,²² and stress is often more prevalent among those with low socioeconomic status (SES).¹²⁶ Stress may also lead to mental health outcomes, such as depression.¹²⁷ During pregnancy, psychosocial stress has been associated with many adverse pregnancy outcomes, including preterm birth. Among women who experience stress during pregnancy, those with high psychosocial stress are at approximately a 25-60% increased risk for preterm birth when compared to those with low levels of psychosocial stress.¹²⁶ Thus, understanding the origins and interrelationships between different aspects of stress in pregnancy is essential to develop successful interventions and for future research on pregnancy outcomes.

Limited research exists regarding which factors may contribute to or coincide with increased psychosocial stress during pregnancy. Some studies of pregnant women have shown that women reporting lower perceived neighborhood safety and increased perceived neighborhood disorder also have high levels of perceived stress, depression²³ and anxiety¹²⁸ and that increased stressful life events, perceived stress, and a lack of social support are associated with increased symptoms of depression.^{127,129,130} Pregnant women who experience intimate partner violence (IPV) are also more likely to experience anxiety and depression compared to those who did not experience IPV.¹³¹ In a non-pregnant population, the relationship between perceived stress and depression varied based on participants' levels of social support.¹³²

Although studies have speculated about associations and explored different links between perceived stress, depression, stressful life events, social support, and the neighborhood context, these associations have not been explored among Puerto Ricans. Pregnant women residing in Puerto Rico may be at a heightened risk for psychosocial stress, as an estimated 10% of Puerto Ricans experience major depressive disorder.¹³³ Although few studies examining psychosocial stress among Puerto Ricans on the island exist,^{133,134} a study of Hispanics subgroups in the mainland U.S. found that the prevalence of depression was highest among Puerto Ricans.¹³⁵ Puerto Ricans residing in the Continental US also experience more socioeconomic stressors, including living in poverty, at greater rates than other Hispanic subgroups and non-Hispanic populations.¹³⁶

The purpose of this study was to examine the associations between depression, social support, and psychosocial stress and their interrelationships among pregnant

women in the Puerto Rico Testsite for Exploring Contamination Threats (PROTECT) population. We created a conceptual model which we tested using a path analysis in order to examine the pathways through which psychosocial stress may influence depression. We also assessed whether social support was directly associated with psychosocial stress, depression and its potential buffering effect.

Methods

Study Population

Pregnant women included in the present study were enrolled in the PROTECT cohort, an ongoing prospective birth cohort in Northern Puerto Rico. PROTECT has been previously described in detail.¹¹³ Briefly, we included a subset of women who were recruited between January 2011 and September 2017 between 14 and 20 weeks gestation from 5 prenatal clinics in the Northern Karst region of Puerto Rico. Women were eligible for inclusion in PROTECT if they were between 18-40 years of age, lived in the Northern Karst region, did not use oral contraceptives 3 months prior to conception, did not have *in vitro* fertilization to become pregnant, and were free of known obstetric and medical complications (e.g., diabetes).¹¹³ Women in PROTECT are invited to complete 3 study visits, targeted at approximately 20 $\pm$ 2 weeks gestation, 24 $\pm$ 2 weeks gestation, and 28 $\pm$ 2 weeks gestation. The Institutional Review Board at all participating locations (University of Michigan, University of Puerto Rico, Northeastern University, University of Georgia) approved PROTECT and all women provided written informed consent prior to participation in the study.

Life Experiences Survey (LES)

Women completed the Life Experiences Survey (LES) at the 2nd study visit, which provided information on whether or not they had experienced certain life events (N=39) anytime in the past year.¹¹⁸ If they did experience the event, they were asked if it had a negative or positive impact, ranging from extremely negative (a score of -3) to extremely positive (a score of +3). The number of events perceived as negative (coded -3, -2, -1) were summed and the absolute value was taken to create a positive, continuous measure of negative life experiences (range 0-26); thus, higher scores were indicative of increased negative life events. Events perceived as positive (a score of +1, +2, +3) or having no impact (a score of 0) were coded as 0 and did not influence the current analysis.

Neighborhood Perceptions (NP)

Also at the 2nd study visit, women were asked two questions about perceptions of their neighborhood. Women were first asked if in their opinion, their neighborhood was a very good (a score of 1), good (a score of 2), not very good (a score of 3), or not at all a very good (a score of 4) place to live. Women were then asked if they felt as if their neighborhood was very safe (a score of 1), somewhat safe (a score of 2), somewhat unsafe (a score of 3), or very unsafe (a score of 4). These questions were adapted from the National Children's Study.¹²² Responses to both questions were summed to create an overall continuous measure of neighborhood perceptions (NP; range 2-8); thus, higher scores were indicative of negative neighborhood perceptions.

Perceived Stress Scale (PSS)

The Perceived Stress Scale (PSS) was administered during the 3rd visit. The PSS is composed of 10 questions and is designed to measure the extent to which

individuals feel that situations in his or her life are stressful.¹²³ Each item asked about how often specific feelings or thoughts, such as feeling nervous or irritated, occurred within the last month. Responses to each question were ranked on a 5 point Likert scale, with responses ranging from “never” (a score of 0) to “almost always” (a score of 4). Some questions that were positively stated, such as successfully dealing with life hassles, were reverse coded so that higher scores were always associated with increased perceived stress. Responses were summed to create a continuous measure of perceived stress (range 0-40), where higher scores were indicative of increased stress.

Center for Epidemiologic Studies-Depression (CES-D)

The 20-item Centers for Epidemiologic Studies-Depression (CES-D) scale was also administered at the 3rd visit. The CES-D is a screening tool measuring depression symptoms according to the Diagnostic Statistical Manual-IV.¹¹⁹ Questions are designed to measure how often in the past week individuals experience depressive symptoms. Responses are ranked on a Likert scale and range from “rarely” (a score of 0) to “majority” (a score of 3). Responses were summed to allow for continuous analysis of the depression scale (range 0-48). Higher scores were consistent with increased feelings of depression.

ENRICHD Social Support Instrument (ESSI)

The Enhancing Recovery in Coronary Heart Disease Patients (ENRICHD) Social Support Instrument (ESSI) was administered during the 3rd visit. The ESSI is a 7-item scale which measures functional social support and was originally created for use in the ENRICHD trial.¹²⁰ Women were asked about amount and sources of social support,

such as having someone available to listen or provide advice, responses ranged from “none of the time” (a score of 1) to “all the time” (a score of 5). Responses were summed to create a continuous measure of social support (range 8-35), where higher scores were indicative of higher social support.

Statistical Analysis

We examined the means and standard deviations (SD) of the CES-D, ESSI, PSS, LES, and NP across demographic characteristics. For each scale, the overall score was coded as missing if the response to any individual question was missing. Linear regression models were used to determine differences in the CES-D, ESSI, PSS, LES, and NP scales across demographic groups. To examine correlations between psychosocial stress measures, we calculated Pearson’s correlation coefficients.

Our conceptual path model was developed by reviewing the literature and previously published research. All continuous measures were assessed for normality. Path analyses were used to test our hypotheses using the package ‘lavaan’¹³⁷ in R Version 3.5.0. Path analysis is an extension of regression analysis which evaluates mediation and estimates standardized regression coefficients reflecting the direct, indirect, and total effects among variables. Direct effects indicate the association between two variables where the effect is not mediated through other included variables. Indirect effects show the relationship between one variable and another, through one or more mediating variables. The total effect is the sum of the direct and indirect effects.

The best fitting version of the model was developed through an iterative process where we tested multiple pathways, starting with two variables and gradually adding

others. We removed those pathways that were non-significant and resulted in poor model fit. Model fit was examined using the chi-square to degree of freedom index (X^2/df ; values <3 are preferred), Root Mean Square Error of Approximation (RMSEA; values <0.05 are preferred), Standardized Root Mean Square Residual (SRMR; values <0.08 are preferred), Comparative Fit Index (CFI; values >0.9 are preferred), and Tucker-Lewis Index (TLI; values >0.9 are preferred).¹³⁸

When calculating standard errors (SE), we used bias-corrected bootstrapping with 1,000 draws and corresponding 95% confidence intervals (CI). Missing data in path analyses were analyzed using the full information maximum likelihood (FIML) estimation, which is a recommended way of handling missing data in structural equation modeling.¹³⁹ FIML is built into the 'lavaan' package and estimates a likelihood function for all participations based on the non-missing CES-D, ESSI, PSS, LES, and NP measures and covariates for each participant so that all available participants and data are used.

To test the hypothesis that social support would moderate the associations between psychosocial stress measures, we used model 58 in the PROCESS macro for SAS 9.4 developed by Hayes.¹⁴⁰ The PROCESS software is a tool for estimating interactions and the conditional indirect effects of moderated moderation models.¹⁴⁰ Continuous variables were mean centered for moderated-mediation analyses. We calculated regression coefficients for associations between psychosocial stress measures among those who experienced low (one SD below the mean ESSI value; simple slope a_1), medium (mean ESSI value), and high (one SD above the mean ESSI value; simple slope a_2) social support. PROCESS model 58 allows for multiple

mediators and provides 5,000 bootstrapped sample estimates for estimation of indirect effects and 95% bias-corrected bootstrapped CIs. P-values <0.05 were considered statistically significant. A complete case analysis (N=841) was used for moderated mediation models.

Results

There were 1,047 women who had information on one or more of the LES, NP, PSS, CES-D, and ESSI scales and were included in these analyses (Table 4.1). The highest percentage of women in the PROTECT analytic sample were between ages 18-24 years (38.0%), had received a college degree (43.6%), were employed (62.4%), and were married (56.4%) (Table 4.1). Significant correlations were observed between all the CES-D, PSS, LES, NP, and ESSI measures (p-value <0.05 for each correlation) (Table 4.2). Scores on the PSS, CES-D, LES, and NP were all positively correlated with one another. The strongest correlation observed was between PSS and CES-D ($r=0.65$; p-value<0.05). The ESSI was inversely correlated with each measure, as expected.

Distribution of missingness on the ESSI, PSS, CES-D, LES, and NP scales across demographic characteristics is provided in Table 4.3. Mean scores on the PSS, LES, and CES-D, scales were higher among women who were between ages 18-24, single, currently drinking alcohol, or ever smokers compared to reference groups (Figures 4.1-4.3). Women with higher stress as measured by NP scale were more likely to be unemployed compared to employed, ever compared to never smokers, and have public compared to private insurance (Figure 4.4). Women with lower scores on the ESSI (indicative of increased stress) were more likely to be unemployed, single or living with a partner, current or ever smokers, and have public insurance compared to

reference groups (Figure 4.5). Overall, most psychosocial stress variables were associated with lower SES indicators.

Marital status, education, and maternal age were *a priori* included as covariates in our path analyses based on their known associations with psychosocial stress.^{130,131} According to our final conceptual model, CES-D was the primary outcome and the exposures that demonstrated associations that were greatest in magnitude included the PSS ($\beta=0.57$, direct path) and the LES ($\beta=0.18$, indirect path through PSS) (Figure 4.6; Table 4.4). In other words, a one SD increase in perceived stress was directly associated with a 57% SD increase in feelings of depression and a one SD increase in negative life experiences was indirectly associated with a 18% SD increase in feelings of depression. Only the PSS and LES were directly associated with the CES-D.

LES was associated with the CES-D through both direct ($\beta=0.15$) and indirect ($\beta=0.18$) paths, and the indirect effect was greater in magnitude than the direct effect. The LES also had a positive direct effect on the PSS ($\beta=0.32$).

NP affected the PSS directly ($\beta=0.12$) and indirectly through LES ($\beta=0.03$). NP also affected the CES-D indirectly ($\beta=0.10$) through its effects on PSS and LES scores. Our final model has good fit, as indicated by the model fit statistics all being within the acceptable range. For example, the RMSEA value was 0.00 and the X^2/df index was 0.71. All paths in our final conceptual model were statistically significant ($p<0.05$ for all paths).

The ESSI was not directly or indirectly associated with the PSS, NP, LES, or CES-D and thus was not included in our final conceptual model. However, the ESSI significantly moderated the relationship between the PSS and CES-D in our conceptual

model. No statistically significant moderation by the ESSI was observed for other relationships. To interpret the moderation finding between PSS and CES-D, we plotted estimated levels of CES-D among those with high, medium, and low ESSI scores (Figure 4.7). Under the condition of low ESSI scores, the indirect effect of NP on CES-D through PSS was greater in magnitude (simple slope $a_1=0.85$, 95% CI=0.76, 0.94) than compared to women with high ESSI scores (simple slope $a_2=0.72$, 95% CI=0.64, 0.82).

Discussion

Our study found evidence that pregnant women in Puerto Rico with high perceived stress have high levels of depression. We also found evidence that neighborhood perceptions influence depression through two separate pathways: 1) through increasing negative life experiences and 2) through the path of increasing perceived stress. To our knowledge, this is the first study examining the associations between these different parameterizations of psychosocial stress in Puerto Rico among pregnant women.

Our study supports a growing body of literature suggesting that the qualities of one's neighborhood may be a source of increased psychosocial stress.^{18,23,141} In our study, neighborhood perceptions were positively associated to all other metrics of psychosocial stress, including negative life experiences, perceived stress and depression. This is in line with previous work showing that women in neighborhoods with high material and social deprivation have increased perceived stress and depression.¹⁸ It is also consistent with a study of African-American women in Michigan showing that lower levels of perceived neighborhood safety and walkability were associated with increased feelings of perceived stress and depression.²³ In addition,

Witt et al¹⁴¹ found that women in disadvantaged neighborhoods experience more stressful life events during pregnancy compared to women in advantaged neighborhoods, which is supported by our findings. Limited research on the positive effects of the neighborhood context exist. However, one study showed that individuals in neighborhoods considered to have high social affluence, residential stability, and neighborhood advantage were less likely to have diabetes.¹⁴²

The direct effect we observed between perceived stress and depression was the greatest in magnitude compared to all other associations in our final model. In addition to a strong direct effect, perceived stress partially mediated the relationships between other psychosocial stress measures (neighborhood perceptions, negative life experiences) and depression. These findings are supported by previous work which demonstrated that perceived stress mediates the relationships between different forms of psychosocial stress and depression.¹⁴³ For example, among African-American women in Detroit, perceived stress mediated the relationship between perceived neighborhood quality and depression.¹⁴³

According to the stress buffering hypothesis, social support may improve psychological well-being by attenuating the impacts of stressful situations.¹⁴⁴ In our study population, social support moderated the relationship between perceived stress and depression, which is consistent with some literature.¹³² However, we found no evidence that social support moderated the preceding associations in our conceptual model. Prior research has suggested that in order for social support to be effective, it needs to be tailored to the experiences of stress.¹⁴⁵ For example, social support specifically tailored to racial discrimination buffered the effects of discrimination on

depression in a population of African-American women.¹⁴⁶ In that study, general social support did not produce these same buffering effects.¹⁴⁶ This may be one explanation for our null findings as the ESSI is a measure of functional social support and is not tailored to the stress experience.

Our finding of little moderation of these stress relationships by social support may also be explained by the low levels of depression and other psychosocial stress indicators in our study. For example, in the PROTECT population, the mean CES-D score was 11.7 (SD=9.13) and the mean PSS score was 14.9 (SD=6.78). Among women enrolled in the Boston Puerto Rican Health Study, the mean CES-D score was 24.4 (SD=9.2), which is markedly increased compared to those in PROTECT.¹⁴⁷ Similar high scores on the CES-D (mean score of 21.8 [SD=7.5]) were observed among a convenience sample of women recruited from primary care clinics in San Juan, PR.¹³⁴ Among women enrolled in the Pregnancy Study Online, the mean PSS score was 15.8 (SD=5.7), which is slightly higher than the mean PSS score in PROTECT.¹⁴⁸ Overall, it seems that pregnant women in Puerto Rico have lower levels of stress than their counterparts in the continental U.S. Importantly, despite PROTECT women experiencing less psychosocial stress, the relationships observed in our path analysis between different parameterizations of psychosocial stress, social support, and depression that have been observed in other studies were still observed in our study population.

Our results should be interpreted in light of its limitations. First, some of our measures were obtained at the same study visit and we are unable to determine temporality. The path analysis assumes directionality and direction of causality of

reporting may be a concern in our study. For example, it is possible that women with depression perceive certain life experiences as more negative.¹⁴⁹ However, all associations we identified in our path analysis have been observed in other studies, giving us confidence in our results. Additionally, only one measure included in our study, the CES-D, is a clinical screening tool. The CES-D is designed to capture symptoms that are correlated with depression.¹¹⁹ PROTECT did not include diagnostic mental health measures, which are the gold standard for determining mental health.

Despite these limitations, our study has many strengths. Importantly, we examined several different types of psychosocial stress, each of which has been used in many other studies. In the creation of final model, we explored several different pathways through which psychosocial stress measures have been associated with one another in the literature, giving us confidence in our results. Finally, PROTECT also employs a prospective study design allowing for future follow up with participants, which is a methodological advancement over previous cross-sectional and retrospective studies exploring the associations between psychosocial stress measures.^{23,129}

Conclusions

Our study highlights the complexity of the relationships between different indices of psychosocial stress among pregnant women in Puerto Rico. Findings from our path analysis indicate that women with negative neighborhood perceptions experience more negative life experiences and have high perceived stress. We also found evidence that women who experience more negative life experiences and have high perceived stress have increased symptoms of depression. Future research investigating stress

parameterizations in relation to adverse maternal and child health outcomes should explicitly consider the mediating and moderating pathways we identified.

Acknowledgements

This work was supported by the National Institute of Environmental Health Sciences grants P42ES017198 and P50ES026049 and the National Institutes of Health Office of the Director grants UG3OD023251 and UH3OD023251.

Table 4.1. Demographic characteristics of study population (N=1,047).

Categorical	N (%)	
Maternal Age, years		
	18-24	397 (38.0)
	25-29	320 (30.6)
	30-34	214 (20.5)
	≥35	115 (11.0)
Maternal Education		
	<High school	77 (7.44)
	High school or equivalent	132 (12.8)
	Some college or technical school	375 (36.2)
	≥College degree	451 (43.6)
Employment Status		
	Unemployed	388 (37.6)
	Employed	644 (62.4)
Pre-pregnancy BMI		
	Underweight (<18.5 kg/m ²)	64 (6.46)
	Normal (18.5-<25 kg/m ²)	492 (49.7)
	Overweight (25-<30 kg/m ²)	262 (26.5)
	Obese (≥30 kg/m ²)	172 (17.4)
Marital Status		
	Single	210 (20.3)
	Married	585 (56.4)
	Living together	242 (23.3)
Alcohol Use		
	Never	524 (51.0)
	Before pregnancy	442 (43.0)
	Current	62 (6.03)
Smoking		
	Never	873 (84.2)
	Before pregnancy	132 (12.7)
	Current	32 (3.09)
Insurance Status		
	Public	364 (35.7)
	Private	637 (62.5)
	Uninsured	19 (1.86)
Continuous		Mean (SD)
	Social Support	27.6 (3.53)
	Perceived Stress	13.7 (6.84)
	Depression	11.6 (9.08)
	Negative Life Experiences	3.02 (4.03)
	Neighborhood Perceptions	2.53 (0.84)

Abbreviations: SD, standard deviation; BMI, body mass index.

Note: numbers may not sum to 1,047 due to missing values

Table 4.2. Pearson correlations between psychosocial stress measures.

	Depression	Perceived Stress	Negative Life Experiences	Social Support	Neighborhood Perceptions
Depression		0.65	0.37	-0.26	0.14
Perceived Stress			0.34	-0.29	0.17
Negative Life Experiences				-0.17	0.09
Social Support					-0.16
Neighborhood Perceptions					

Note: all correlations are significant at p value <0.05

Table 4.3. Distribution of missingness between demographic characteristics and psychosocial stress measures.

	Missing Social Support (N=86)	Missing Perceived Stress (N=78)	Missing Depression (N=109)	Missing Negative Life Experiences (N=71)	Missing Neighborhood Perceptions (N=25)
	N (%)	N (%)	N (%)	N (%)	N (%)
Maternal Age, years					
18-24	28 (32.6)	27 (34.6)	44 (40.4)	35 (49.3)	13 (52.0)
25-29	22 (25.6)	22 (28.2)	25 (22.9)	21 (29.6)	6 (24.0)
30-34	19 (22.1)	19 (24.4)	24 (22.0)	8 (11.3)	4 (16.0)
≥35	17 (19.8)	10 (12.8)	16 (14.7)	7 (9.86)	2 (8.00)
Maternal Education					
<High school	6 (7.79)	9 (11.8)	11 (10.4)	9 (10.0)	1 (4.00)
High school or equivalent	11 (14.3)	10 (13.2)	12 (11.3)	13 (18.8)	6 (24.0)
Some college or technical school	26 (33.8)	28 (36.8)	42 (39.6)	23 (33.3)	10 (40.0)
≥College degree	34 (44.2)	29 (38.2)	41 (38.7)	24 (34.8)	8 (32.0)
Employment Status					
Unemployed	28 (36.4)	31 (40.8)	44 (41.1)	31 (44.3)	15 (60.0)
Employed	49 (63.6)	45 (59.2)	63 (58.9)	39 (55.7)	10 (40.0)
Pre-pregnancy BMI					
Underweight (<18.5 kg/m ²)	5 (6.58)	7 (9.33)	8 (7.62)	8 (12.3)	4 (17.4)
Normal (18.5-<25 kg/m ²)	36 (47.4)	36 (48.0)	49 (46.7)	28 (43.1)	9 (39.1)
Overweight (25-<30kg/m ²)	23 (30.3)	20 (26.7)	28 (26.7)	17 (26.2)	4 (17.4)
Obese (≥30kg/m ²)	12 (15.8)	12 (16.0)	20 (19.0)	12 (18.5)	6 (26.1)
Marital Status					
Single	21 (27.6)	25 (32.9)	23 (21.5)	14 (20.0)	4 (16.0)
Married	40 (52.6)	37 (48.7)	56 (52.3)	35 (50.0)	12 (48.0)
Living together	15 (19.7)	14 (18.4)	28 (26.2)	21 (30.0)	9 (36.0)
Alcohol Use					
Never	38 (49.4)	39 (51.3)	52 (48.6)	37 (53.6)	17 (68.0)
Before pregnancy	31 (40.3)	30 (39.5)	48 (44.9)	27 (39.1)	7 (28.0)
Current	8 (10.4)	7 (9.21)	7 (6.54)	5 (7.25)	1 (4.00)

Smoking						
	Never	64 (83.1)	61 (80.3)	86 (80.4)	59 (84.3)	20 (80.0)
	Before pregnancy	10 (13.0)	11 (14.5)	16 (15.0)	9 (12.9)	3 (12.0)
	Current	3 (3.90)	4 (5.26)	5 (4.67)	2 (2.86)	2 (8.00)
Insurance Status						
	Public	31 (38.9)	37 (51.4)	52 (51.0)	24 (51.1)	0 (0.00)
	Private	46 (57.5)	31 (43.1)	47 (46.1)	22 (46.8)	1.00 (100.0)
	Uninsured	3 (3.75)	4 (5.56)	3 (2.94)	1 (2.13)	0 (0.00)

Abbreviations: BMI, body mass index

Table 4.4. Standardized regression coefficients for best fitting structural equation model.

Negative Life Experiences			
	Direct (SE)	Indirect (SE)	Total (SE)
Neighborhood Perceptions	0.08 (0.15)	-	0.08 (0.15)
Perceived Stress			
Neighborhood Perceptions	0.12 (0.25)	0.03 (0.08)	0.15 (0.27)
Negative Life Experiences	0.32 (0.06)	-	0.32 (0.06)
Depression			
Neighborhood Perceptions	-	0.10 (0.08)	0.10 (0.08)
Negative Life Experiences	0.15 (0.07)	0.18 (0.05)	0.34 (0.08)
Perceived Stress	0.57 (0.04)	-	0.57 (0.04)

Note: all paths are significant at p value < 0.05 ; standard errors are estimated using 1,000 bootstrap estimates; missing data handled using full information maximum likelihood specification; - indicates no path; model adjusted for maternal age, marital status, and maternal education.

Abbreviations: SE, standard error

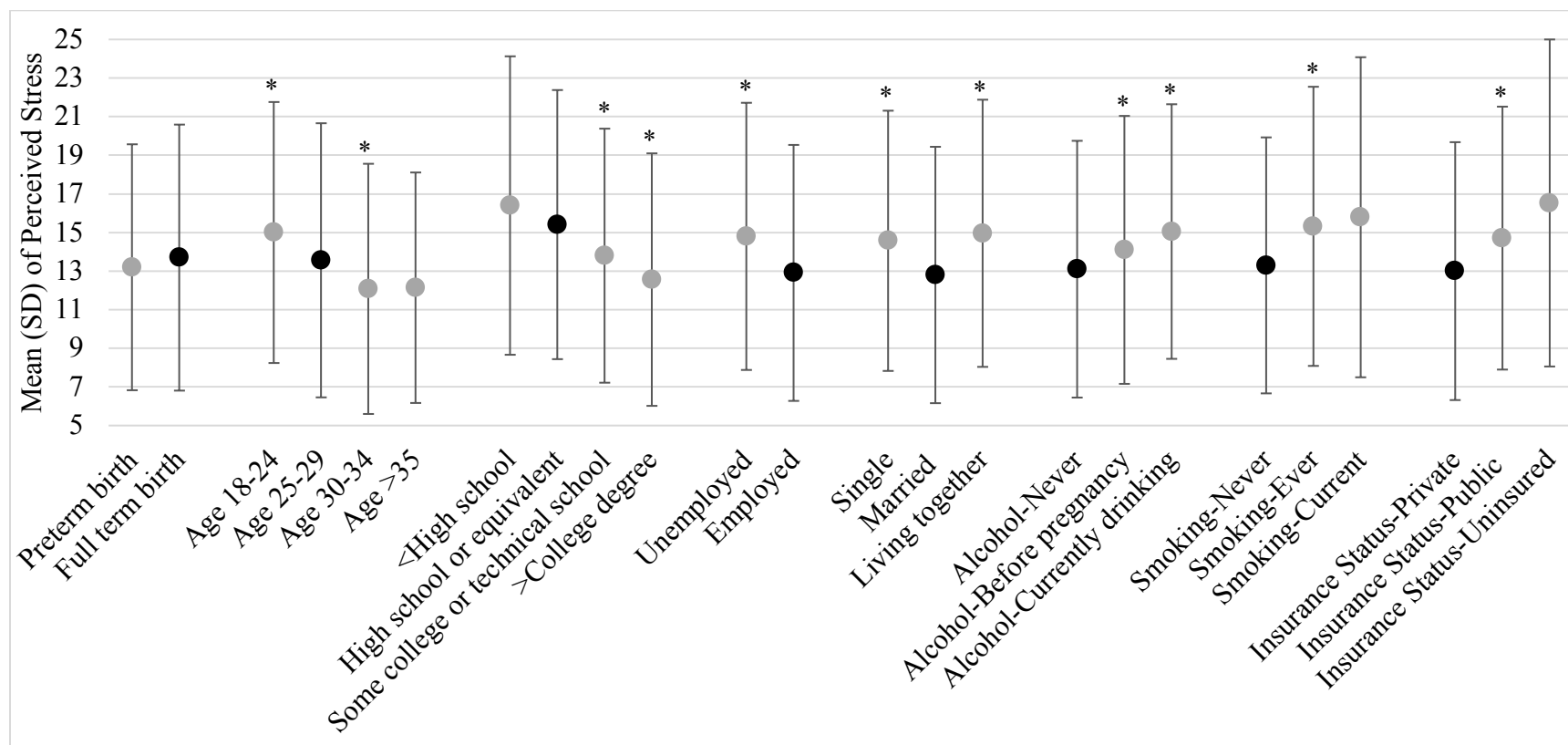


Figure 4.1. Distribution of perceived stress across demographic characteristics.

*Indicates p-value < 0.05

Abbreviations: SD, standard deviation

Note: black indicates reference levels; p-values calculated from linear models

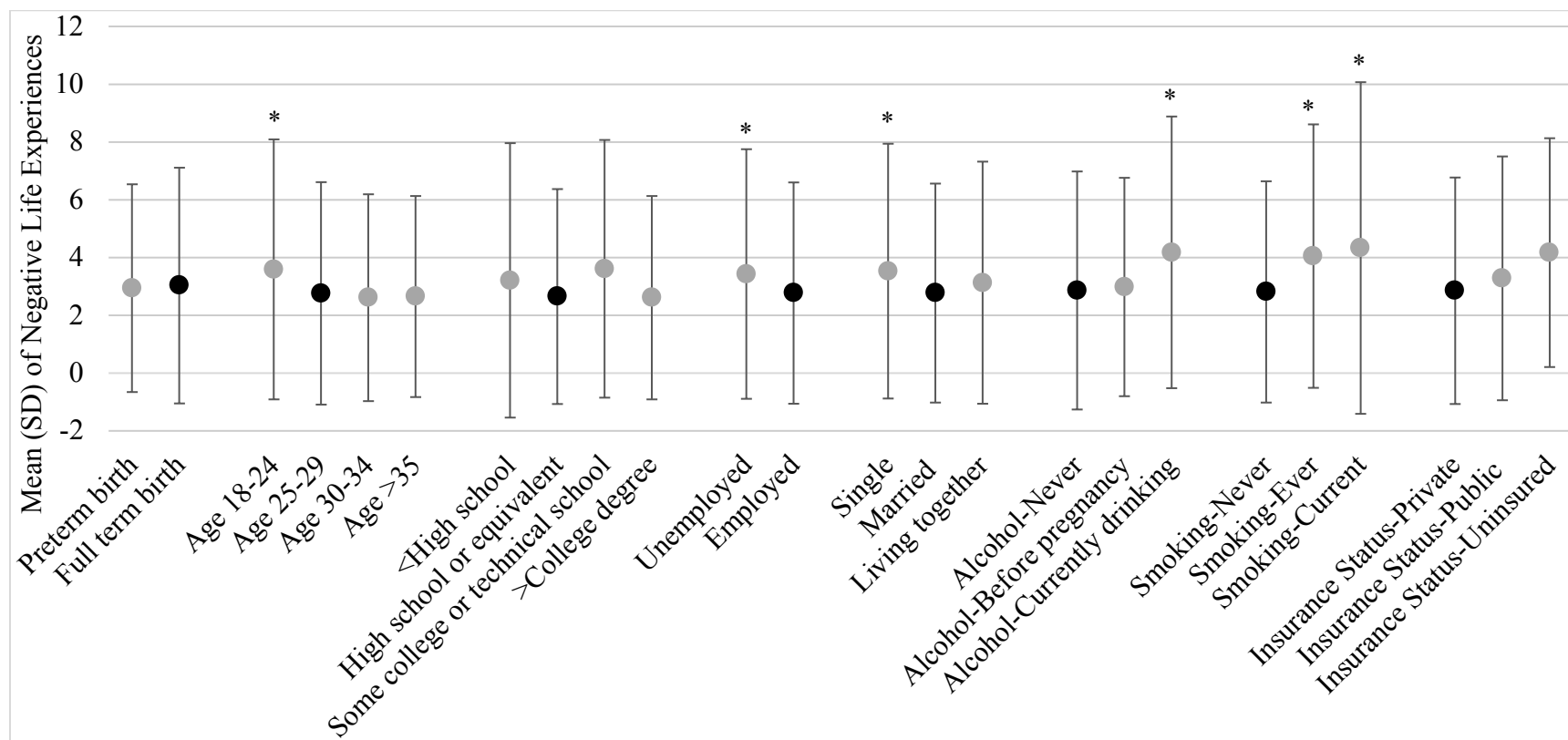


Figure 4.2. Distribution of negative life experiences across demographic characteristics.

*Indicates p-value < 0.05

Abbreviations: SD, standard deviation

Note: black indicates reference levels; p-values calculated from linear models

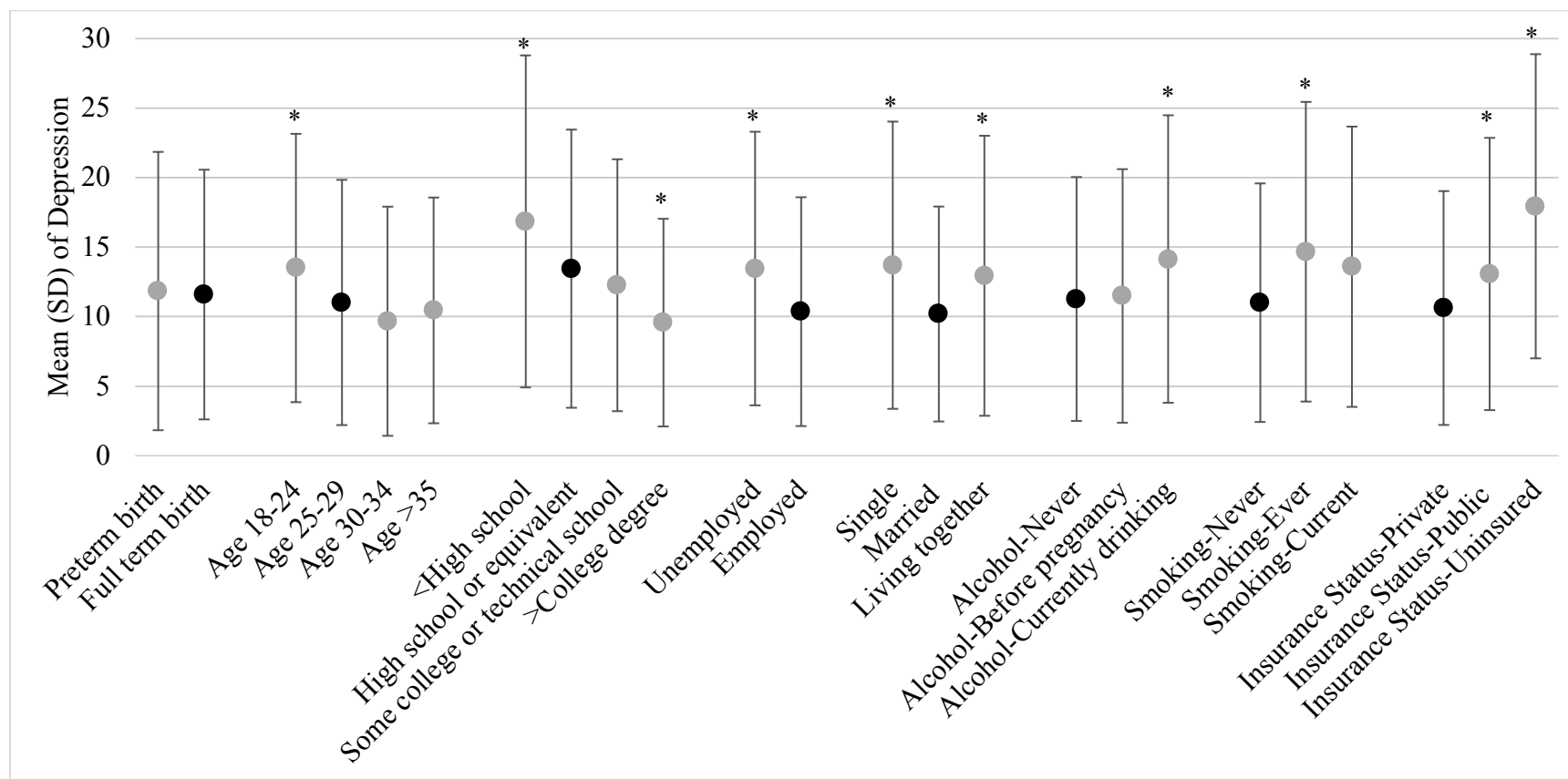


Figure 4.3. Distribution of depression across demographic characteristics.

*Indicates p-value < 0.05

Abbreviations: SD, standard deviation

Note: black indicates reference levels; p-values calculated from linear models

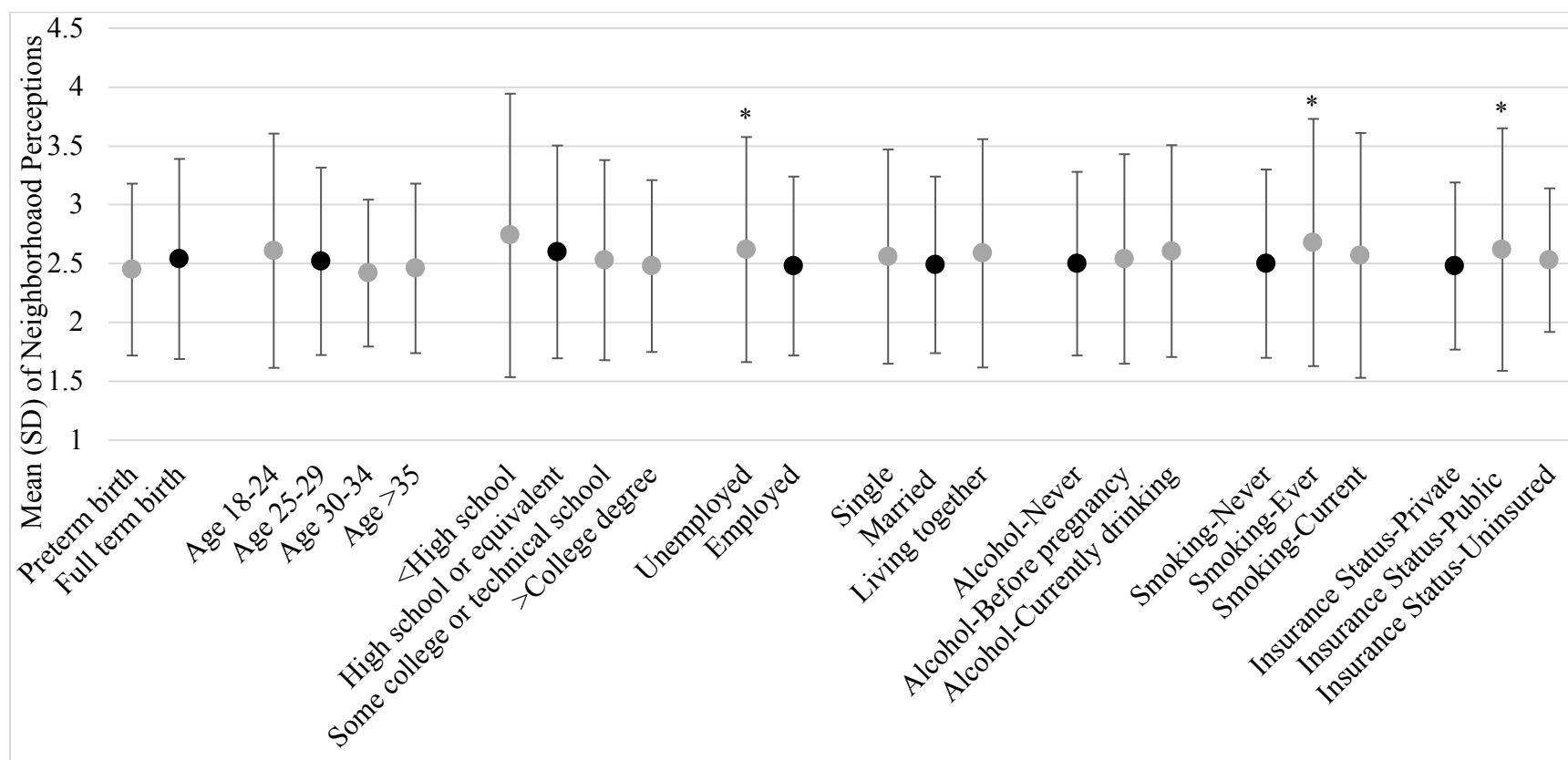


Figure 4.4. Distribution of neighborhood perceptions across demographic characteristics.

*Indicates p-value < 0.05

Abbreviations: SD, standard deviation

Note: black indicates reference levels; p-values calculated from linear models

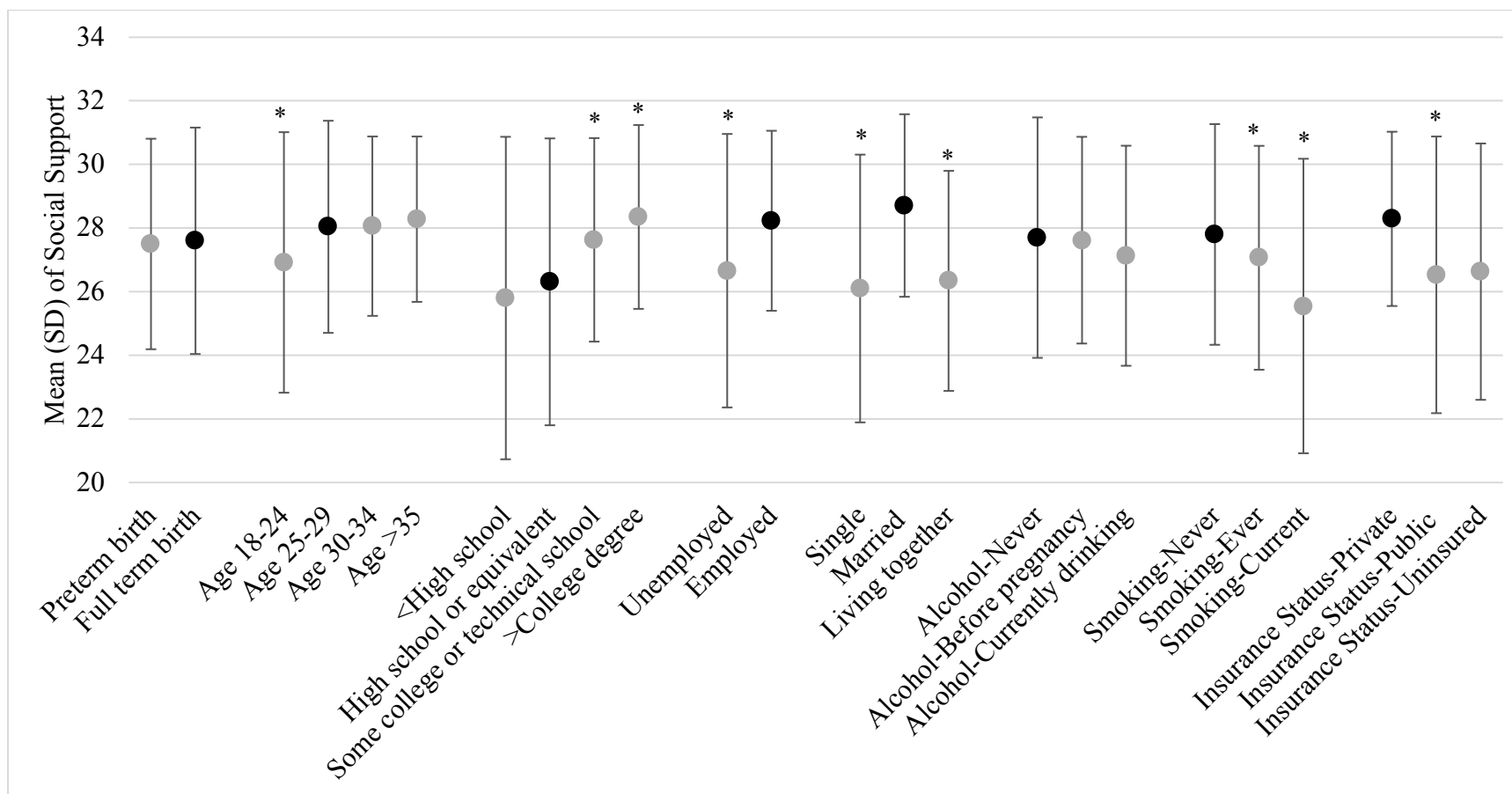


Figure 4.5. Distribution of social support across demographic characteristics.

*Indicates p-value < 0.05

Abbreviations: SD, standard deviation

Note: black indicates reference levels; p-values calculated from linear models.

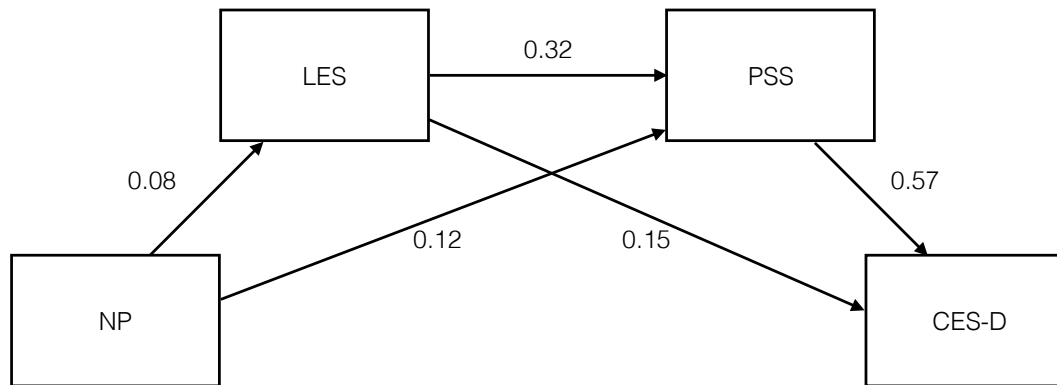


Figure 4.6. Path diagram indicating the relationship between psychosocial stress measures in PROTECT study population. Maternal age, marital status, and education are included as covariates in model (N=1,033). Note: All paths are significant at $p < 0.05$; missing data handled using full information maximum likelihood. Model fit statistics: $X^2 = 0.71$, p value=0.40, CFI=1.00, TLI, 1.02, RMSEA=0.00, SRMR= 0.00. Abbreviations: NP, neighborhood perceptions; LES, life experience survey; PSS, perceived stress scale; CES-D, center for epidemiologic studies-depression; X^2/df , chi-square to degree of freedom index; RMSEA, Root Mean Square Error of Approximation; CFI, Comparative Fit Index; TLI, Tucker-Lewis Index; SRMR, Standardized Root Mean Square Residual

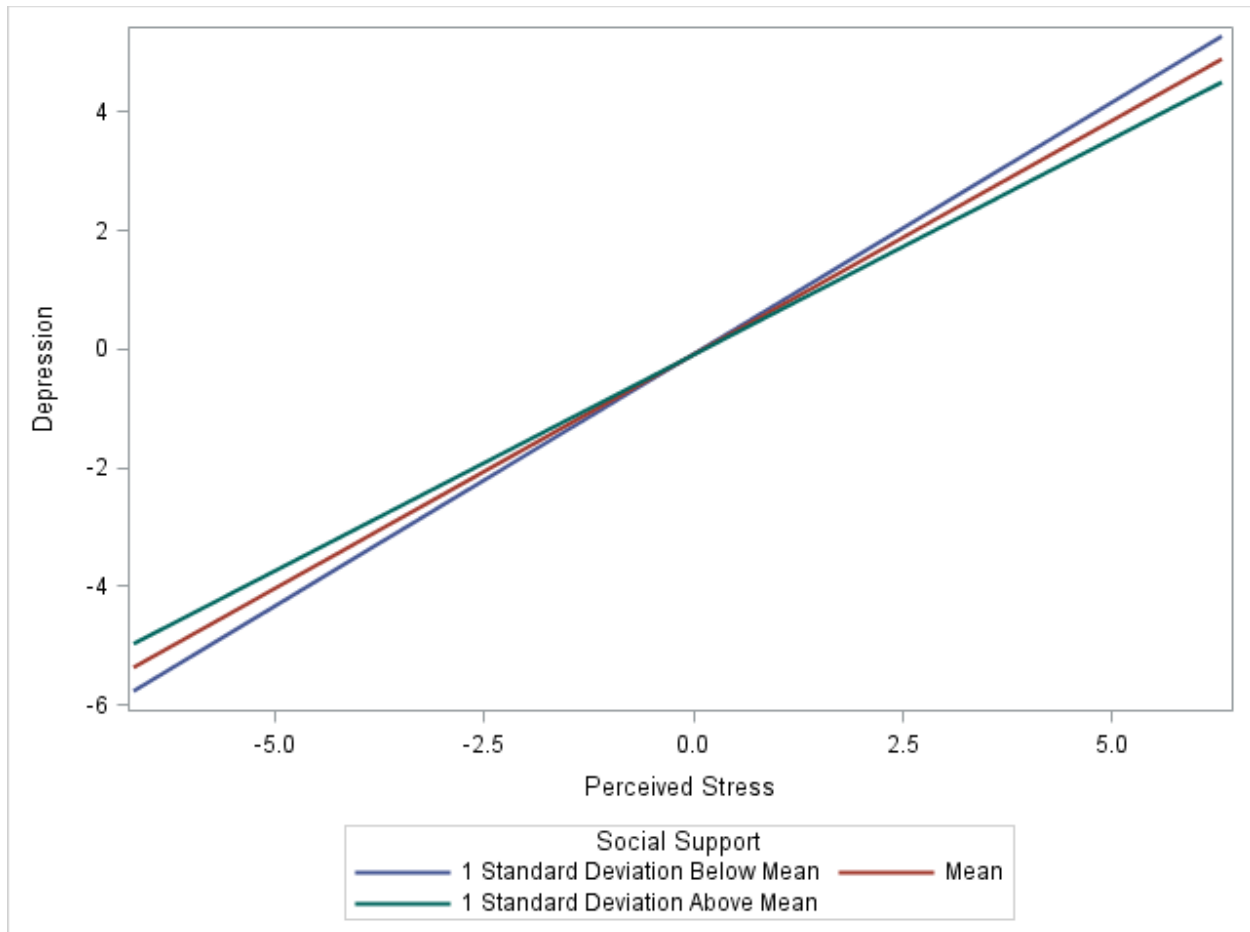


Figure 4.7. Effect of perceived stress on depression moderated by social support. Abbreviations: CES-D; center for epidemiologic studies-depression; PSS, perceived stress scale; ESSI, ENRICHD social support instrument.

CHAPTER 5

ASSOCIATIONS BETWEEN PSYCHOSOCIAL STRESS AND OXIDATIVE STRESS DURING PREGNANCY IN NORTHERN PUERTO RICO

Background: Psychosocial stress during pregnancy has been associated with preterm birth. Oxidative stress may be one pathway linking the two. In this study, we examined associations between self-reported psychosocial stress measures during pregnancy and urinary oxidative stress biomarker concentrations among pregnant women, hypothesizing that women with increased psychosocial stress would have elevated oxidative stress.

Methods: Perceived stress, depression, negative life experiences, neighborhood perceptions, and social support were included as measures of psychosocial stress during pregnancy. Each was assessed using validated questionnaires administered at the 2nd and 3rd study visits. Responses were grouped into tertiles for analysis, where the highest tertile corresponded to highest stress. Urinary concentrations of 8-isoprostane, its major metabolite, and prostaglandin-F-_{2α} were measured at the 1st, 2nd, and 3rd study visits and averaged across pregnancy as biomarkers of oxidative stress or inflammation. Linear models were used to examine crude and adjusted associations between tertiles of psychosocial stress and each oxidative stress biomarker.

Results: The geometric mean of 8-isoprostane, its metabolite, and prostaglandin-F-_{2α} was higher among women who were between 18-24 compared to 25-29 years of age, who had some college education compared to a college degree or higher, and were

unemployed compared to employed. In adjusted analyses, no associations were observed between any indices of psychosocial stress and oxidative stress biomarker concentrations.

Conclusions: Psychosocial stress during pregnancy was not associated with oxidative stress biomarkers in our study population. However, results from our study suggest that oxidative stress biomarkers are increased among women of lower socio-economic status. Psychosocial stress levels in PROTECT may be lower than stress levels in other populations.

Introduction

The rate of preterm birth in Puerto Rico is among the highest both in the U.S. and globally.³ In 2006, the rate of preterm birth in Puerto Rico was 19.9% and although this rate decreased to 11.5% in 2017, it remains high relative to the continental U.S.^{5,150} Psychosocial stress may be a risk factor for preterm birth in Puerto Rico, as previous studies have shown that psychosocial stress during pregnancy is associated with preterm birth.¹⁷

Despite these established associations between psychosocial stress and preterm birth, the mechanism linking psychosocial stress to preterm birth remains unknown. One pathway may be through activation of the hypothalamic-pituitary-adrenal (HPA) axis. Activation of the HPA axis can lead to increased cortisol production, in turn prematurely activating corticotropin-releasing hormone, which is associated with an increased risk of preterm birth.⁵⁵

Oxidative stress represents another potential mechanism.⁵⁵ Oxidative stress, commonly measured using the biomarker 8-isoprostane,¹³ is the imbalance between the

amount of reactive oxygen species and the ability of the antioxidant species to neutralize them. Oxidative stress has been associated with a variety of pregnancy complications, including preeclampsia and preterm birth.^{15,16} Studies in non-pregnant populations have shown that oxidative stress is increased among individuals who experience extreme stressful life events, depression, anxiety, and higher perceived stress compared to low stress individuals.^{10,14,151} During pregnancy, the relationships between psychosocial stress and oxidative stress remain largely unknown. However, previous research has identified suggestive associations between extreme stressful life events, anxiety, depression and urinary 8-isoprostane.¹⁵²

The objective of the current study was to investigate the associations between five measures of psychosocial stress during pregnancy and biomarkers of urinary oxidative stress concentrations using data from the Puerto Rico Testsite for Exploring Contamination Threats (PROTECT) pregnancy cohort. We hypothesized that women with increased psychosocial stress would have elevated levels of oxidative stress biomarkers during pregnancy. Additionally, we *a priori* hypothesized that the relationship between psychosocial stress measures and oxidative stress biomarker concentrations would vary based on the amount of social support one has, as previous studies have shown that social support moderates the associations between the stressor and the stress response.¹⁵³

Methods

Study Population

Women included in this study are a subset of women enrolled in the PROTECT cohort. PROTECT is an ongoing, prospective cohort study and methods have been

previously described in detail elsewhere.^{113,154} Briefly, women were recruited from prenatal clinics in the Northern Karst region of Puerto Rico. Women included in this analysis delivered between August 2012 and April 2017, prior to Hurricane Maria. Exclusion criteria for PROTECT included: maternal age less than 18 or greater than 40 years of age, use of *in vitro* fertilization to become pregnant, oral contraceptives use for 3 months prior to pregnancy, existing obstetric and medical complications (e.g., diabetes). Spot urine samples are provided at each study visit and demographic information is obtained via questionnaire at the first study visit. All women provided written, informed consent and the Institutional Review Board at all participating locations (University of Puerto Rico, University of Georgia, Northeastern University, University of Michigan) approved this study.

Psychosocial Stress

Life Experience Survey (LES)

The Life Experiences Survey (LES) was administered at the 2nd study visit. The LES asks if certain life events (n=39) have occurred anytime in the past year.¹¹⁸ Women were asked to rate the impact of these life events as ranging from extremely negative (a score of -3) to extremely positive (a score of +3). We summed the number of events perceived as negative (coded -3, -2, -1) and took the absolute value. This created a continuous measure of negative life experiences (range 0-28). We coded the events perceived as positive (a score of +1, +2, or +3) or having no impact (a score of 0) as 0. As such, they had no impact on the current analysis.

Neighborhood Perceptions (NP)

Women were asked two questions about perceptions of neighborhood safety and quality adapted from the National Children's Study at the 2nd study visit.¹²² The first question was: "In your opinion your neighborhood is a ..." and responses ranged from "very good" to "very poor" (a score of 1 and 4, respectively). The second question was: "Do you feel that your neighborhood is a ..." and responses ranged from "very safe" to "very unsafe" (a score of 1 and 4, respectively). Responses to both questions were summed (range 2-7) to create an overall continuous measure of negative neighborhood perceptions (NP).

Perceived Stress Scale (PSS)

The 10-item Perceived Stress Scale (PSS) was administered at the 3rd visit. The PSS is designed to measure how individual feels about stressful situations by asking about specific thoughts, such as feeling nervous or irritated.¹²³ Responses on the PSS ranged from "never" (a score of 0) to "almost always" (a score of 4) and were summed to create a continuous measure (range 0-40). Questions that were positively stated, such as successfully dealing with life hassles, were reverse coded so that higher scores on individual questions and the overall scale were always associated with increased perceived stress.

Center for Epidemiologic Studies-Depression (CES-D)

The 20-item Center for Epidemiologic Studies-Depression (CES-D) scale was administered at the 3rd study visit.¹¹⁹ The CES-D is a screening tool designed to measure symptoms of depression occurring within the past week. Responses on the CES-D ranged from "rarely" (a score of 0) to "majority" (a score of 3) and were summed to create a continuous measure of depression symptomology (range 0-46).

ENRICHD Social Support Instrument (ESSI)

Lastly, the Enhancing Recovery in Coronary Heart Disease Patients (ENRICHD) Social Support Instrument (ESSI) was administered at the 3rd visit. The ESSI is a 7-item scale which measures social support and was originally designed for use in the ENRICHD trial but is appropriate for use in the general population. Questions on the ESSI asks about the amount and availability of social support. Responses ranged from “none of the time” (a score of 1) to “all the time” (a score of 5). Responses to individual questions on the ESSI were summed to create a continuous measure of social support (range 1-33).

Psychosocial stress questionnaires were administered in either English or Spanish by trained study staff. Higher scores on all psychosocial stress measures were indicative of increased psychosocial stress. The ESSI is an exception, where lower scores on the ESSI are indicative of increased stress. If the response to any individual question on the scale was missing, the overall scale was coded as missing. For ease of interpretation, psychosocial stress measures were grouped into tertiles (i.e., low, medium, high stress) for analyses.

Oxidative Stress Biomarker Assessment

Methods for collecting and processing urine samples have been previously described.¹⁵⁵ Briefly, spot urine samples were collected in polypropylene containers and divided into aliquots. Samples were frozen –80 °C and remained frozen until analysis. Urinary specific gravity (SpG) was measured using a digital handheld refractometer to indicate urine dilution.

The Eicosanoid Core Laboratory at Vanderbilt University Medical Center (Nashville, TN) analyzed free 8-isoprostane, its major metabolite, and prostaglandin-F-_{2α} as biomarkers of oxidative stress or inflammation using stable isotope dilution gas chromatography-negative ion chemical ionization-mass spectrometry in 476 samples (N= 272 at visit 1, N= 345 at visit 2, N= 221 at visit 3). This method requires a C18 column for solid-phase extraction, a thin-layer chromatography purification, and chemical derivation. During analyses, samples are thawed and 0.25 ml urine is diluted in 10 ml pH 3 water and acidified to pH 3 using 1N HCl.¹⁵⁶ Further details describing measurement of oxidative stress concentrations are available elsewhere.¹⁵⁶

For all urinary oxidative stress biomarkers, values below the limit of detection (LOD) were replaced by LOD/the square root of 2. All urinary oxidative stress concentrations were corrected for SpG using the equation $Ox_c = Ox[(1.019-1)/(SpG-1)]$, where the median SpG was 1.019 in the PROTECT population. Ox is the measured oxidative stress concentration and Ox_c is the specific-gravity corrected measure. We then took the geometric mean of the available SpG corrected oxidative stress concentrations across visits. This was done to obtain a more stable estimate of oxidative stress. For example, if a participant had 8-isoprostane measured at each visit, we took the geometric mean of SpG-corrected 8-isoprostane at visits 1, 2, and 3. If a participant had SpG-corrected 8-isoprostane at only the 1st visit, we used only that measure. All SpG-corrected oxidative stress concentrations were log transformed for normality.

Statistical Analysis

Frequencies and counts were used to describe the PROTECT study population. Geometric means and geometric standard deviations (SD) were used to examine distributions of oxidative stress biomarker concentrations. Linear regression models were used to calculate crude and adjusted beta estimates and 95% confidence intervals (CI) for the associations between psychosocial stress in tertiles (low, medium, high stress; Table 5.1) and oxidative stress biomarker concentrations. Beta estimates were converted to % difference in oxidative stress biomarker concentration for ease of interpretation, e.g., for individuals with high and medium compared to low psychosocial stress. QQ-plots were examined for each model to ensure assumptions of linear regression were met. Tests for linear trend using tertile psychosocial stress measures were conducted using the Cochran Armitage test.¹⁵⁷

We included maternal age in years (18-24, 25-29, 30-34, ≥ 35), maternal education (<high school, high school degree or equivalent, some college or technical school, $\geq$ college degree), employment status (unemployed, employed), alcohol use (never, before pregnancy, current), smoking (never, before pregnancy, current), marital status (single, married, living together and unmarried), and insurance status (public, private, uninsured) as covariates in our analyses. Maternal age in years was modeled categorically in regression models for consistency with previous work in this cohort.¹⁵⁴ Marital status, education, and maternal age were *a priori* included as covariates in final adjusted models based on their known associations with psychosocial stress.^{130,131} We additionally used a forward selection approach, where covariates that changed point estimates by greater than 10% were retained in final adjusted models.

We explored possible effect measure modification by ESSI by examining associations between tertiles of psychosocial stress (PSS, CES-D, LES, NP) and 8-isoprostane, 8-isoprostane metabolite, and prostaglandin-F_{2α} concentrations within strata of ESSI (medium and high stress tertiles versus low stress tertile). Medium and high levels of the ESSI were combined because clear differences between these groups were not seen. We also tested the inclusion of an interaction term for 3 level psychosocial stress*2 level ESSI in the overall model using ANOVA. P-values for interaction terms <0.10 were considered statistically significant. As a sensitivity analysis, we also examined associations between psychosocial stress measures and oxidative stress biomarker concentrations at visit 3 only to ensure that oxidative stress concentrations measured after psychosocial stress (e.g., reverse causality) was not a concern.

Missing data for psychosocial stress measures and covariates was handled using the multiple imputation via chained equations (MICE) approach. MICE was implemented using the 'mice' package in R to impute missing values for psychosocial stress measures and covariates using conditionally specified models. Oxidative stress biomarker concentrations were not included as predictors in the imputation procedure. Ten values were produced for each observation with missing data and results from each imputation were pooled for analyses. All analyses were conducted in R Version 3.5.0 and SAS 9.4 (Cary, NC).

Results

There were 476 women in our analysis who had urine samples measured for oxidative stress biomarker concentrations. Most women included in this analysis were

between 18-24 years of age, married or living with a partner, employed, and had a college degree or higher (Table 5.2). In bivariate analyses, the geometric mean of 8-isoprostane and its metabolite was higher among women who were between 18-24 compared to 25-29 years of age and women who were unemployed compared to employed. Similarly, the geometric mean of 8-isoprostane, its metabolite, and prostaglandin-F_{2α} was higher among women with less education compared to women with a college degree or higher. Compared to women with private insurance, the geometric mean of 8-isoprostane, its metabolite, and prostaglandin-F_{2α} was lower among women with public insurance (Table 5.2).

Maternal age, maternal education, and marital status were included as covariates in final adjusted models. In adjusted analyses, no measure of psychosocial stress was associated with 8-isoprostane or prostaglandin-F_{2α} (Table 5.3). For all indices of psychosocial stress with the exception of the LES, high compared to low psychosocial stress was associated with a modest but non-significant increase in the 8-isoprostane metabolite (Table 5.3). For example, high compared to low scores on the PSS were associated with a 3.05% non-significant increase in the 8-isoprostane metabolite (95% CI=-6.57%, 13.7%). Similarly, low compared to high scores on the ESSI (indicative of increased stress) were associated with a 3.67% non-significant increase in the 8-isoprostane metabolite (95% CI=-7.29%, 15.9%) Tests for trend were also non-significant for all associations between psychosocial stress and oxidative stress biomarker concentrations. In crude analyses, low compared to high scores on the ESSI was significantly associated with a 11.6% increase in the 8-isoprostane metabolite (95%

CI=1.01, 23.4) (Table 5.4). No associations were observed between other psychosocial stress indices and oxidative stress biomarkers in crude analyses (Table 5.4).

No evidence of interaction was observed using the ANOVA test and associations between psychosocial stress and oxidative stress biomarkers were similar within strata of the ESSI (Table 5.5). Notably, among women in the medium and high tertiles of the ESSI, high compared to low psychosocial stress was associated with a modest, non-significant increase in oxidative stress biomarker concentrations across all psychosocial stress measures and biomarkers examined. For example, high compared to low scores on the CES-D were associated with a non-significant 8.65% increase in 8-isoprostane (95% CI=-2.64%, 21.3%) among women in the medium and high tertiles of the ESSI. The corresponding % difference in 8-isoprostane among women in the lowest tertile of the ESSI was -1.98% (95% CI=-19.4, 1.92.)

Association between tertiles of psychosocial stress and oxidative stress biomarkers measured at the 3rd study visit were similar to results including the entire sample and no clear trends were observed (Table 5.6).

Discussion

In this study, we examined associations between five measures of psychosocial stress and oxidative stress biomarkers during pregnancy. Our hypothesis was that increased psychosocial stress during pregnancy would be associated with elevated levels of oxidative stress biomarker concentrations. Overall, our findings suggest psychosocial stress is not associated with increased oxidative stress biomarkers in the PROTECT cohort.

We included perceived stress, depression, negative life experiences, neighborhood perceptions, and social support as psychosocial stress indicators in this study. Including these diverse parameterizations of psychosocial stress is an important strength of this study. In the PROTECT population, only low compared to high social support was associated with elevated oxidative stress biomarker concentrations, specifically the 8-isoprostane metabolite. Although this is a significant association, this finding may be a result of multiple testing as we observed no association between social support and the 8-isoprostane metabolite in adjusted analyses and or with other oxidative stress biomarkers. Nonetheless, this finding is consistent with previous research showing in adjusted analyses that increasing workplace social support is associated with decreased oxidative stress, specifically 8-hydroxy-2'-deoxyguanosine.¹⁵⁸

There were no statistically significant associations detected between perceived stress, depression, negative life experiences, neighborhood perception and oxidative stress biomarker concentrations in our study. However, the trends were in the expected direction, where women with high compared to low scores on the depression scale had increased concentrations of oxidative stress as measured by all biomarkers. Furthermore, the percent increase in oxidative stress biomarkers among women with high compared to low scores on the depression scale was generally greater in magnitude than associations among women with medium compared to low scores on this scale. Although this finding did not reach statistical significance, it is consistent with previous research from another pregnancy cohort suggesting that depression is associated with increased oxidative stress.^{10,11}

Studies in non-pregnant populations have identified an association stressful life events and oxidative stress biomarkers, specifically isoprostanes.¹⁵⁹ Similarly, our previous work in an multi-center pregnancy cohort found an association between family death, an extreme stressful life event, and free 8-isoprostane in unadjusted analyses.¹⁵² Although this finding was not confirmed in the PROTECT population, we did find that women with high compared to low scores on the LES had marginally elevated 8-isoprostane levels. It is possible that we did not observe any associations due to our lack of power. Given our current sample size, we have very limited power to detect differences in oxidative stress biomarkers.

Our null associations may also be due to the PROTECT study population experiencing less psychosocial stress relative to other populations. Some of our previous work with these psychosocial stress measures in the PROTECT population has shown that the mean levels of psychosocial stress are lower than mean stress levels observed in other cohorts. In PROTECT, the mean CES-D and PSS score was 11.7 and 14.9, respectively. In comparison, the mean CES-D score among women in the Boston Puerto Rico Health Study was 24.4.¹⁴⁷ Similarly, a study of women recruited from primary care clinics in San Juan, PR reported that the mean CES-D score was 21.8.¹³⁴ Lastly, the mean PSS score among women enrolled in the Pregnancy Study Online, a prospective cohort study examining factors related to fertility, was 15.8.¹⁴⁸ It is possible that only extremely stressful situations would result in changes in oxidative stress and thus lower stress populations, such as PROTECT, would be less susceptible to psychosocial stress-related changes in oxidative stress.

In addition to 8-isoprostane, we also measured its metabolite which is hypothesized to be a more sensitive biomarker than 8-isoprostane in urine.¹⁶⁰ In our study, associations with psychosocial stress and the metabolite were somewhat greater in magnitude and in the expected directions relative to 8-isoprostane. The metabolite is generally formed from blood levels in the lungs, unaffected by direct kidney synthesis,¹⁶¹ and thus may be more reflective of concentrations of whole body concentrations. Although no adjusted associations between psychosocial stress and the metabolite reached statistical significance, the sensitivity of the 8-isoprostane metabolite may represent one possible explanation for why the associations we observed with the metabolite were slightly greater in magnitude.

Our results should be interpreted in light of some limitations. Although we included many measures of psychosocial stress in our study, our scales only measured stress immediately before and during pregnancy. We had no measure of psychosocial stress prior to pregnancy. A woman's reproductive potential may be modified by both early life experiences, such as stressful life events, and cumulative allostatic load. It is possible that psychosocial stress measured across the life course is more relevant in this context.¹⁶²

Despite these limitations, our study has many strengths. First, we examined multiple indices of psychosocial stress, which allowed us to examine many different types of stress and explore associations that have been observed in other studies. Second, we included multiple biomarkers of oxidative stress, allowing us to look at oxidative stress in different ways and to examine many biologically relevant associations. Importantly, the biomarker 8-isoprostane is thought to be one of the best

biomarkers of oxidative stress because it is stable, including during human pregnancy, unaffected by lipids in the diet, and is detectable in urine.^{61,163,164}

Conclusions

In the PROTECT study population, psychosocial stress was not associated with oxidative stress biomarkers. However, the associations between depression and negative life experiences were in the expected directions. Results from our study indicate that oxidative stress biomarker concentrations are higher among women of lower socio-economic status. In our study population, the levels of psychosocial stress are lower than stress levels observed in other studies. Future research should explore other environmental factors that may be associated with increased oxidative stress levels in this population.

Acknowledgements

This work was supported by the NIH National Institute of Environmental Health Sciences grants P42ES017198 and P50ES026049 and the National Institutes of Health Office of the Director grants UG3OD023251 and UH3OD023251 and award U54 MD007600 from the National Institute on Minority Health and Health Disparities at NIH.

Table 5.1. Distribution of psychosocial stress measures in tertiles.

	Lowest Tertile	Middle Tertile	Highest Tertile
	Range	Range	Range
Social Support	0-27	28-29	>29
Perceived Stress	0-9	10-16	>16
Depression	0-6	7-12	>12
Negative Life Experiences	0	1-3	>3
Neighborhood Perceptions	0-2	3	>3

Table 5.2. Distribution of subject specific averages of urinary oxidative stress biomarkers (ng/mL) corrected for specific gravity by demographic characteristics.

Characteristic	8-isoprostane (N=476)			8-isoprostane metabolite (N=476)			Prostaglandin-F _{2α} (N=476)		
	N	Geometric Mean (Geometric SD)	p	N	Geometric Mean (Geometric SD)	p	N	Geometric Mean (Geometric SD)	p
Maternal Age									
18-24	189	2.13 (1.49)	<0.01	189	1.04 (1.52)	<0.01	189	3.11 (1.63)	<0.01
25-29	148	1.80 (1.47)	Ref	148	0.84 (1.51)	Ref	148	2.64 (1.70)	Ref
30-34	85	1.78 (1.50)	0.83	85	0.83 (1.50)	<0.01	85	2.68 (1.58)	0.82
>35	54	1.66 (1.43)	0.19	54	0.85 (1.49)	<0.01	54	2.47 (1.57)	0.39
Maternal Education									
<High school	35	2.01 (1.41)	0.06	35	0.99 (1.42)	<0.01	35	3.04 (1.68)	0.11
High school or equivalent	72	2.09 (1.58)	<0.01	72	1.04 (1.60)	<0.01	72	2.91 (1.63)	0.15
Some college or technical school	171	1.99 (1.51)	<0.01	171	0.97 (1.56)	<0.01	171	2.94 (1.67)	0.03
≥College degree	198	1.75 (1.45)	Ref	198	0.81 (1.45)	Ref	198	2.62 (1.63)	Ref
Employment Status									
Unemployed	295	2.01 (1.51)	0.02	295	0.99 (1.54)	<0.01	295	2.89 (1.73)	0.30
Employed	181	1.84 (1.48)	Ref	181	0.87 (1.51)	Ref	181	2.75 (1.60)	Ref
Marital Status									
Single	92	1.94 (1.44)	0.23	92	0.95 (1.50)	<0.01	92	2.77 (1.60)	0.89
Married	256	1.83 (1.49)	Ref	256	0.85 (1.52)	Ref	256	2.74 (1.67)	Ref
Living together	128	2.02 (1.52)	0.03	128	1.02 (1.52)	<0.01	128	2.97 (1.63)	0.14
Alcohol Use									
Never	209	1.94 (1.47)	Ref	209	0.93 (1.51)	Ref	209	2.71 (1.66)	Ref
Before pregnancy	242	1.88 (1.50)	0.46	242	0.90 (1.54)	0.48	242	2.91 (1.63)	0.13
Currentl	25	1.81 (1.62)	0.43	25	0.90 (1.56)	0.71	25	2.72 (1.29)	0.97
Smoking									
Never	393	1.87 (1.48)	Ref	393	0.90 (1.53)	Ref	393	2.76 (1.66)	Ref
Before pregnancy	64	2.05 (1.58)	0.09	64	0.96 (1.57)	<0.01	64	3.07 (1.60)	0.11
Current	19	2.10 (1.31)	0.24	19	0.93 (1.32)	<0.01	19	2.86 (1.53)	0.78
Insurance Status									
Private	293	2.06 (1.49)	Ref	293	1.01 (1.50)	Ref	293	3.11 (1.66)	Ref
Public	177	1.81 (1.48)	<0.01	177	0.85 (1.53)	<0.01	177	2.64 (1.63)	<0.01

Uninsured	6	2.29 (1.36)	0.15	6	1.15 (1.38)	<0.01	6	2.60 (1.43)	0.94
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Abbreviations: SD, standard deviation Ref, reference.

Table 5.3. Adjusted¹ associations between subject specific averages of urinary oxidative stress biomarkers (ng/mL) corrected for specific gravity and psychosocial stress.

	8-isoprostane (N=476)	8-isoprostane metabolite (N=476)	Prostaglandin-F- _{2α} (N=476)
	% Difference (95% CI)	% Difference (95% CI)	% Difference (95% CI)
Social Support			
High	Ref	Ref	Ref
Medium	-3.92 (-12.9, 5.97)	0.30 (-9.86, 11.4)	3.05 (-10.2, 18.2)
Low	-2.96 (-12.0, 7.04)	3.67 (-7.29, 15.9)	-5.82 (-17.9, 8.03)
p trend	0.55	0.64	0.37
Perceived Stress			
Low	Ref	Ref	Ref
Medium	-4.88 (-12.1, 2.88)	0.60 (-8.25, 10.3)	-1.98 (-12.9, 10.3)
High	-1.98 (-11.1, 8.11)	3.05 (-6.57, 13.7)	-1.00 (-12.0, 11.4)
p trend	0.68	0.56	0.87
Depression			
Low	Ref	Ref	Ref
Medium	-1.00 (-10.2, 9.20)	0.00 (-8.71, 9.76)	2.02 (-7.50, 12.5)
High	3.05 (-6.57, 13.7)	0.03 (-7.13, 13.4)	4.08 (-7.47, 17.1)
p trend	0.49	0.61	0.46
Negative Life Experiences			
Low	Ref	Ref	Ref
Medium	4.08 (-5.64, 14.8)	-0.04 (-12.6, 5.56)	2.94 (-8.12, 15.3)
High	7.25 (-2.76, 18.3)	0.00 (-9.43, 9.75)	0.50 (-10.3, 12.6)
p trend	0.16	0.87	0.88
Neighborhood Perceptions			
Low	Ref	Ref	Ref
Medium	0.30 (-8.35, 9.76)	0.01 (-8.42, 11.4)	-9.52 (-19.6, 1.78)
High	-1.00 (-11.6, 10.9)	0.02 (-9.30, 14.8)	-1.00 (-13.7, 13.6)
p trend	0.91	0.75	0.39

¹Models are adjusted for maternal age, maternal education, and marital status
Abbreviations: CI, confidence interval; Ref, reference.

Table 5.4. Crude associations between subject specific averages of urinary oxidative stress biomarkers (ng/mL) corrected for specific gravity and psychosocial stress.

	8-isoprostane (N=476)	8-isoprostane metabolite (N=476)	Prostaglandin-F- 2 α (N=476)
	% Difference (95% CI)	% Difference (95% CI)	% Difference (95% CI)
Social Support			
High	Ref	Ref	Ref
Medium	1.41 (-6.97, 10.5)	8.00 (-1.31, 18.2)	5.13 (-6.54, 18.3)
Low	3.05 (-6.21, 13.2)	11.6 (1.01, 23.4)	-1.98 (-12.9, 10.3)
p trend	0.53	0.02	0.83
Perceived Stress			
Low	Ref	Ref	Ref
Medium	-2.66 (-10.9, 6.31)	4.08 (-5.64, 14.8)	-1.00 (-12.0, 11.4)
High	3.05 (-6.21, 13.2)	9.42 (-0.80, 20.7)	3.05 (-8.39, 15.9)
p trend	0.63	0.09	0.65
Depression			
Low	Ref	Ref	Ref
Medium	6.18 (-3.16, 16.4)	1.01 (-8.42, 11.4)	2.02 (-9.30, 14.8)
High	8.33 (-1.20, 18.8)	8.33 (-1.78, 19.5)	7.25 (-4.65, 20.6)
p trend	0.14	0.13	0.21
Negative Life Experiences			
Low	Ref	Ref	Ref
Medium	8.33 (-1.78, 19.5)	-1.24 (-10.3, 8.67)	3.05 (-8.30, 15.9)
High	6.18 (-3.73, 17.1)	2.01 (-7.53, 12.5)	2.02 (-9.30, 14.8)
p trend	0.07	0.74	0.64
Neighborhood Perceptions			
Low	Ref	Ref	Ref
Medium	-1.19 (-9.71, 8.13)	-0.30 (-0.25, 9.54)	-10.4 (-20.4, 0.76)
High	1.31 (-9.75, 13.7)	4.50 (-7.46, 18.0)	2.02 (-11.1, 17.0)
p trend	0.94	0.57	0.54

¹Models are adjusted for maternal age, maternal education, and marital status

Abbreviations: CI, confidence interval; Ref, reference

Table 5.5. Adjusted¹ associations between subject specific averages of urinary oxidative stress biomarkers (ng/mL) corrected for specific gravity and psychosocial stress stratified by social support.

	8-isoprostane			8-isoprostane metabolite			Prostaglandin-F _{2α}		
	Low Social Support (N=126)	Medium/High Social Support (N=350)	p ²	Low Social Support (N=126)	Medium/High Social Support (N=350)	p ²	Low Social Support (N=126)	Medium/High Social Support (N=350)	p ²
	% Difference (95% CI)	% Difference (95% CI)		% Difference (95% CI)	% Difference (95% CI)		% Difference (95% CI)	% Difference (95% CI)	
Perceived Stress			0.66			0.43			0.94
Low	Ref	Ref		Ref	Ref		Ref	Ref	
Medium	-17.3 (-33.3, 2.59)	-2.96 (-12.0, 7.04)		-14.8 (-32.7, 7.81)	3.25 (-6.94, 14.6)		-7.69 (-29.8, 21.5)	-2.96 (-12.0, 21.5)	
High	-11.3 (-27.1, 7.90)	2.02 (-9.30, 14.8)		-3.92 (-23.6, 19.2)	2.33 (-9.20, 15.3)		-5.82 (-27.0, 21.5)	2.02 (-9.30, 14.8)	
p trend	0.43	0.85		0.98	0.65		0.72	0.85	
Depression			0.33			0.69			0.36
Low	Ref	Ref		Ref	Ref		Ref	Ref	
Medium	1.01 (-18.6, 25.3)	-0.20 (-1.19, 0.80)		-5.82 (-22.6, 14.6)	2.02 (-7.50, 12.5)		4.08 (-19.3, 34.3)	-0.20 (-9.69, 10.3)	
High	-1.98 (-19.4, 19.2)	8.65 (-2.64, 21.3)		-1.98 (-19.4, 19.2)	4.08 (-7.47, 17.1)		-2.96 (-24.8, 25.2)	8.65 (-2.64, 21.3)	
p trend	0.81	0.18		0.94	0.52		0.75	0.18	
Negative Life Experiences			0.98			0.80			0.81
Low	Ref	Ref		Ref	Ref		Ref	Ref	
Medium	12.8 (-7.32, 37.2)	3.05 (-6.57, 13.7)		-6.76 (-23.4, 13.4)	-2.96 (-13.7, 9.16)		13.9 (-8.20, 41.3)	3.05 (-6.57, 13.7)	
High	12.8 (-7.32, 37.2)	6.18 (-5.60, 19.4)		-6.76 (-23.4, 13.4)	2.02 (-9.30, 14.8)		17.4 (-7.24, 48.5)	6.18 (-5.60, 19.4)	
p trend	0.25	0.29		0.51	0.81		0.17	0.29	
Neighborhood Perceptions			0.03			0.28			0.14
Low	Ref	Ref		Ref	Ref		Ref	Ref	
Medium	12.8 (-5.48, 34.5)	-3.92 (-12.9, 5.97)		12.8 (-5.48, 34.5)	-3.25 (-13.1, 7.77)		16.2 (-6.35, 44.1)	-3.92 (-12.9, 5.97)	
High	-13.1 (-29.9, 7.85)	7.25 (-6.50, 23.0)		1.01 (-17.0, 22.9)	2.12 (-11.8, 18.3)		-11.3 (-31.3, 14.4)	7.25 (-6.50, 23.0)	
p trend	0.44	0.70		0.66	0.96		0.50	0.70	

¹Models are adjusted for maternal age, maternal education, and marital status

²P-value for interaction term corresponding to dichotomous social support*3 level psychosocial stress measure; p-values calculated using ANOVA

Abbreviations: CI, confidence interval; Ref, reference

Table 5.6. Adjusted¹ associations between subject specific averages of urinary oxidative stress biomarkers (ng/mL) at visit 3 corrected for specific gravity and psychosocial stress.

	8-isoprostane (N=221)	8-isoprostane metabolite (N=221)	Prostaglandin-F- _{2α} (N=221)
	% Difference (95% CI)	% Difference (95% CI)	% Difference (95% CI)
Social Support			
High	Ref	Ref	Ref
Medium	8.00 (-9.11, 28.3)	12.8 (-7.32, 37.2)	12.8 (-10.9, 42.7)
Low	1.71 (-14.1, 20.4)	10.5 (-9.15, 34.5)	5.13 (-18.5, 35.6)
p trend	0.88	0.36	<0.01
Perceived Stress			
Low	Ref	Ref	Ref
Medium	-4.88 (-17.1, 9.11)	-6.76 (-21.8, 11.2)	-16.5 (-32.7, 3.62)
High	-2.96 (-15.4, 11.3)	-7.69 (-22.6, 10.1)	-12.2 (-29.2, 8.94)
p trend	0.70	0.36	0.22
Depression			
Low	Ref	Ref	Ref
Medium	-1.98 (-14.6, 12.4)	4.08 (-11.0, 21.8)	-9.52 (-25.6, 10.1)
High	-1.98 (-16.2, 14.7)	-11.3 (-25.7, 5.80)	-13.1 (-29.9, 7.85)
p trend	0.81	0.21	0.20
Negative Life Experiences			
Low	Ref	Ref	Ref
Medium	-1.98 (-14.6, 12.4)	-4.88 (-20.3, 13.5)	-15.6 (-32.0, 4.67)
High	-3.92 (-16.2, 10.2)	-5.82 (-21.1, 12.3)	-11.3 (-28.5, 10.0)
p trend	0.55	0.46	0.20
Neighborhood Perceptions			
Low	Ref	Ref	Ref
Medium	-0.40 (-13.0, 14.0)	1.01 (-13.7, 18.2)	-17.3 (-32.7, 1.64)
High	-3.25 (-18.4, 14.7)	2.02 (-16.1, 24.1)	-0.03 (-22.4, 28.9)
p trend	0.74	0.82	0.49

¹Models are adjusted for maternal age, maternal education, and marital status

Abbreviations: CI, confidence interval; Ref, reference

CHAPTER 6

PSYCHOSOCIAL STRESS AND PRETERM BIRTH IN PUERTO RICO

Background: Preterm birth (PTB), the leading cause of infant morbidity and mortality worldwide, disproportionally impacts pregnancies in Puerto Rico. Psychosocial stress may be an important risk factor for PTB and has not been explored in Puerto Rico.

Methods: We used data from the Puerto Rico Testsite for Exploring Contamination Threats (PROTECT) cohort (N=1,047) to examine associations between psychosocial stress and gestational age continuously and PTB (<37 weeks gestation) using linear and logistic regression, respectively. Psychosocial stress during pregnancy was self-reported at the 2nd and 3rd visits (median 23.4 and 27.1 weeks gestation, respectively) by questionnaires, including the Perceived Stress Scale (PSS), Life Experiences Survey (LES), two questions about neighborhood perceptions (NP), Center for Epidemiologic Studies-Depression Scale (CES-D), and the ENRICH Social Support Instrument (ESSI). Responses on each scale were summed to create continuous measures of stress, which we grouped into tertiles (high, medium, low).

Results: High compared to low psychosocial stress was not associated with gestational age or preterm birth across any of the measures examined. For example, high compared to low scores on the PSS were not associated with gestational age ($\beta=0.21$; 95% confidence interval [CI]=-0.12, 0.55). Similarly, high compared to low scores on the LES were not associated with PTB (odds ratio=1.11; 95% CI=0.64, 1.93).

Conclusions: This is the first study examining psychosocial stress and PTB in Puerto Rico. Distributions of psychosocial stress levels in PROTECT are lower than what has previously been observed in other studies. Our study indicates that psychosocial stress during pregnancy is not a major risk factor for PTB in this population.

Introduction

Preterm birth, one of the leading cause of neonatal morbidity and mortality worldwide,¹ disproportionately impacts pregnancies in Puerto Rico.² The rates of preterm birth in Puerto Rico historically are some of the highest in the U.S. and globally, with rates as high as 19.9% in 2006.⁴ Although this rate has since declined, it remains high relative to the continental U.S.⁵

Maternal psychosocial stress during pregnancy may represent a possible risk factor for preterm birth in Puerto Rico. Studies have shown that psychosocial stress, as indicated by stressful life events,^{22,165} perceived stress,²⁴ depression,¹⁶⁶ or anxiety,²² are increased among women who go on to deliver preterm. Additionally, social support may buffer the effects of the effects of stressful life events by providing a coping mechanism.²¹ The quality of one's neighborhood may also be a source of increased psychosocial stress, specifically depression,¹⁰⁴ and is a risk factor for preterm birth.²³

It is hypothesized that psychosocial stress contributes to preterm birth through activation of the hypothalamic-pituitary-adrenal (HPA) axis, which increases cortisol production.⁸ Psychosocial stress may also increase oxidative stress,¹⁵² which is increased in mothers who go on to experience preeclampsia and preterm birth.^{15,16} Increased exposure to psychosocial stress across the life course may also lead to preterm birth, resulting from increased susceptibility to infection during pregnancy and

immune dysregulation.¹⁶² Additionally, psychosocial stress may lead to unhealthy behaviors, such as smoking or poor nutrition,¹⁶⁷ which may increase the risk of preterm birth through separate pathways.

To date, evidence examining psychosocial stress as a risk factor for preterm birth among Puerto Ricans on the island is lacking. However, among Puerto Ricans in Massachusetts, women with high compared to low perceived stress at mid-pregnancy had increased odds of delivering preterm.²⁴ Puerto Ricans in the mainland may also experience more psychosocial stress than other Hispanic subgroups. For example, Puerto Ricans have a higher prevalence of psychiatric disorders¹⁶⁸ and exhibit higher rates of depression¹³⁵ compared to other Hispanic groups in the mainland U.S. Additionally, Puerto Ricans in the mainland were twice as likely to develop post-traumatic stress disorder following the September 11 terrorist attacks and exhibited lower social support relative to other Hispanic populations.¹⁶⁹

In the present study, we examined associations between five measures of maternal psychosocial stress during pregnancy and gestational age as well as preterm birth using data from the Puerto Rico Testsite for Exploring Contamination Threats (PROTECT) cohort. Perceived stress, depression, negative life experiences, neighborhood perceptions, and social support were included as measures of psychosocial stress. We hypothesized that increased psychosocial stress would be associated with decreased gestational age and increased odds of preterm birth. We additionally hypothesized that social support would modify the associations between individual psychosocial stress measures and gestational age and preterm birth as

previous studies have hypothesized that social support may provide a buffering mechanism.²¹

Methods

Study Population

PROTECT is an ongoing, prospective cohort study and methods have been previously described in detail elsewhere.¹¹³ Women are recruited prior to 20 weeks gestation from affiliated prenatal clinics in the Northern Karst aquifer region. Women are eligible for inclusion in PROTECT if they were between 18-40 years of age, lived in the Northern Karst aquifer region, do not use oral contraceptives for 3 months prior to becoming pregnant, do not use *in vitro* fertilization to become pregnant, and are free of known obstetric and medical complications (e.g., diabetes). At the first visit (timed at 20±2 weeks gestation), women complete a questionnaire containing information on demographic characteristics. Psychosocial stress questionnaires are administered at the 2nd and 3rd study visits (timed at 24±2 and 28±2 weeks gestation, respectively). Women included in this analysis delivered between January 2011 and September 2017 prior to the arrival of Hurricane Maria. The Institutional Review Board at all participating locations (University of Puerto Rico, Northeastern University, University of Michigan, University of Georgia) approved PROTECT and all women provided written, informed consent prior to participating in the study.

Life Experience Survey (LES)

Women completed the Life Experiences Survey (LES) at the 2nd study visit, which provided information on whether or not they had experienced 38 specific life events within the last year.¹¹⁸ For events that had occurred, women were asked to rate

the event as having a positive or negative impact. For all events, responses ranged from extremely negative (a score of -3) to extremely positive (a score of +3). We took the sum of the impact for all events perceived as negative (coded -3, -2, and -1) and took the absolute value to create a positive, continuous measure of negative life experiences (range 0-26); thus, higher scores were indicative of increased negative life events. Events perceived as positive (coded +1, +2, +3) or having no impact (a score of 0) were coded as 0 and thus did not influence the summary score.

Neighborhood Perceptions (NP)

Questions about neighborhood perceptions were adapted from the National Children's Study.¹²² The questions were: "In your opinion your neighborhood is a..." and "Do you feel that your neighborhood is...". Responses to both questions were ranked on a 4 point Likert scale. Responses to the first question ranged from "a very good place to live" (a score of 1) to a "very poor place to live" (a score of 4). On the second question, responses ranged from "very safe" (a score of 1) to "very unsafe" (a score of 4). Responses to each question were summed to create an overall continuous measure of neighborhood perceptions (NP; range 2-8).

Perceived Stress Scale (PSS)

The 10-item Perceived Stress Scale (PSS) was administered during the 3rd visit. The PSS asks about the frequency within the last month of specific negative feelings or thoughts.¹²³ Questions on the PSS that reflect positive feelings, such as successfully dealing with life hassles, were reverse coded so that higher scores on all individual questions reflected higher stress levels. Responses to each question were ranked on a 5 point Likert scale, with responses ranging from "never" (a score of 0) to "almost

always” (a score of 4). Responses were summed to create a continuous measure of perceived stress (range 0-40).

Center for Epidemiologic Studies-Depression (CES-D)

The Centers for Epidemiologic Studies-Depression (CES-D) scale was also administered at the 3rd visit. The CES-D is a 20 question screening tool measuring depression symptoms according to the Diagnostic Structural Manual.¹¹⁹ Questions are designed to measure how often on a given day women experience depressive symptoms. Responses are ranked on a Likert scale and range from “rarely” (a score of 0) to “majority” (a score of 3). Responses were summed to create a continuous measure of depression (range 0-48).

ENRICHD Social Support Instrument (ESSI)

The Enhancing Recovery in Coronary Heart Disease Patients (ENRICHD) Social Support Instrument (ESSI) is a 7-item scale measuring social support and was also administered during the 3rd visit.¹²⁰ Women were asked about amount and sources of social support, such as having someone available to listen or provide advice. Responses to questions on the ESSI ranged from “none of the time” (a score of 1) to “all the time” (a score of 5). Responses were summed to create a continuous measure of social support (range 1-35).

For all psychosocial stress measures, the overall score was coded as missing if the response to any individual question was missing. Higher scores on all scales were indicate of higher stress levels. The ESSI is an exception, where lower scores on the ESSI indicate higher stress. All psychosocial stress measures were subsequently grouped into tertiles (i.e. high, medium, and low stress) for analyses. We also explored

including psychosocial stress continuously and presented categorical results for best interpretability.

Gestational age

Gestational age was assessed using self-reported date of last menstrual period collected at the first study visit and first ultrasound estimates of gestational age per American College of Obstetricians and Gynecologists (ACOG) guidelines.^{154,170}

Gestational age was treated continuously in analyses. We also categorized gestational age into preterm birth (<37 weeks gestational age) and full term birth ($\geq$ 37 weeks gestational age).

Statistical Analysis

Frequencies, counts, means, and standard deviations (SD) were used to describe the demographic characteristics of our study population. Chi-squared tests were used to determine differences between preterm and full term births. Maternal age in years (18-24, 25-29, 30-34, $\geq$ 35) was categorized for consistency with previous work in this cohort.¹⁵⁴ Maternal education (<high school, high school degree or equivalent, some college or technical school, $\geq$ college degree), employment status (unemployed, employed), alcohol use (never, before pregnancy, current), smoking (never, before pregnancy, current), marital status (single, married, living together and unmarried), and insurance status (public, private, uninsured) were also included as covariates in our analysis.

We used linear regression to calculate crude and adjusted beta estimates and 95% confidence intervals (CI) for the associations between tertiles of individual psychosocial stress measures and gestational age. Standard linear regression

assumptions were checked by examining QQ-plots for all models. Logistic regression was used to calculate crude and adjusted odds ratios and 95% CIs for the associations between individual psychosocial stress measures and preterm birth. Tests for linear trend using psychosocial stress measures in tertiles were conducted using the Cochrane-Armitage test.¹⁵⁷ Marital status, education, and maternal age were *a priori* included as covariates in final adjusted models based on their known associations with psychosocial stress.^{130,131}

To assess possible interaction by social support, we examined the adjusted associations between individual psychosocial stress measures and gestational age and preterm birth in models stratified by social support (medium/high vs low social support). Medium and high levels of social support were combined because no clear differences were observed between these groups in other analyses. We also tested the inclusion of an interaction term (2-level social support * 3-level psychosocial stress) using ANOVA. Missing data for psychosocial stress measures and covariates were handled using Multiple Imputation via Chained Equations (mice), in which the independent variables with complete data were used to predict missing values.¹⁷¹ Gestational age and preterm birth were not used as predictors for missing values. We used the package 'mice' in R Version 3.5.0 to produce 10 values for all psychosocial stress measures and covariates with missing values.¹⁷² Statistical analysis was done using R Version 3.5.0 and SAS 9.4 (Cary, NC). Statistical significance was assessed at p-value <0.05. P-values for interaction <0.10 were considered statistically significant.

Results

There were 1,047 women in PROTECT who had gestational age information available and were included in this analysis. There were 107 (10.2%) preterm births in the PROTECT analytic sample (Table 6.1). The mean (SD) of scores on the ESSI, PSS, CES-D, LES, and NP scales was 27.6 (3.53), 13.7 (6.84), 11.6 (9.08), 3.02 (4.03), and 2.53 (0.84), respectively. A greater percentage of women who were between 30-34 years of age (23.4% vs 20.1%), had less than a high school education (12.2% vs 6.95%), were unemployed (47.8% vs 36.7%), and living together but unmarried (32.9% vs 22.3%) delivered preterm relative to women who delivered at term.¹⁵⁴ Few differences were observed between tertiles of psychosocial stress within strata of preterm birth (Table 6.1).

Associations between psychosocial stress and gestational age were null (Table 6.2). In adjusted analyses, women with low compared to high scores on the ESSI had no difference in gestational age ($\beta=0.15$, 95% CI=-0.28, 0.58). High compared to low PSS scores were also not associated with gestational age ($\beta=0.21$, 95% CI=-0.12, 0.55), as was NP ($\beta=0.01$, 95% CI=-0.43, 0.44). No difference in gestational age was observed for women with high compared to low LES values ($\beta=-0.04$, 95% CI=-0.37, 0.30). Similar associations were observed among women with high compared to low CES-D scores ($\beta=0.13$, 95% CI=-0.22, 0.48). Tests for linear trend were non-significant across all psychosocial stress measures.

Associations observed between psychosocial stress measures and preterm birth were similar to those for gestational age (Table 6.3). For example, in adjusted analyses women with high compared to low scores on the PSS had no difference in odds of preterm birth (OR=0.71; 95% CI=0.40, 1.63). A 11% non-significant increase in odds of

preterm birth was observed among women with high compared to low scores on the LES (95% CI=0.64, 1.93). High compared to low scores on the CES-D were not with preterm birth (OR=1.02; 95% CI=0.57, 1.69). As with gestational age, all tests for linear trend were non-significant, indicating no evidence of a dose-response relationship between psychosocial stress and preterm birth in this population.

We found no indication of interaction in models stratified by levels of the ESSI and all interaction terms estimated with ANOVA were non-significant (p-value >0.2 for all models). Within both strata of the ESSI, associations between psychosocial stress measures and preterm birth were similar (Table 6.4). Among women with low social support, no association was observed for high compared to low scores on the LES and preterm birth (OR=1.44, 95% CI=0.52, 4.01). The corresponding OR among women with medium and high social support was 0.89 (95% CI=0.45, 1.76). Associations between psychosocial stress and gestational age within strata of the ESSI were similar to associations seen with preterm birth (data not shown).

Associations between psychosocial stress, gestational age, and preterm birth with unimputed data were similar (data not shown; N=841 for complete case analyses).

Discussion

We examined the relationship between psychosocial stress, gestational age and preterm birth among pregnant women in Northern Puerto Rico. In our study population, perceived stress, depression, negative life experiences, neighborhood perceptions and social support were not statistically associated with gestational age or preterm birth. However, findings from the PROTECT cohort suggest that some demographic characteristics, including those indicative of lower socioeconomic status, such as having

less than a high school education, being unemployed, and having public insurance are more common among women who delivered preterm compared to term.¹⁵⁴

We hypothesized that increased psychosocial stress would be associated with decreased gestational age and increased risk of preterm birth, as this has been observed in other studies and is biologically plausible.^{19,22,24,165} For example, among women in North Carolina, the impact of negative life events for high stress compared to low stress was associated with increased risk of preterm birth (relative risk [RR]=1.8, 95% CI=1.2-2.7).²² Women in Los Angeles who perceived their neighborhoods as poor were 1.3 times more likely to deliver preterm compared to women who perceived their neighborhoods as good (95% CI=1.20-1.41).¹⁹ Among Puerto Rican women in Massachusetts, higher levels of perceived stress during mid-pregnancy were associated with increased odds of delivering preterm.²⁴ However, early pregnancy perceived stress was not associated with preterm birth within this population.²⁴

Although positive associations between psychosocial stress and preterm birth have been observed in other studies,^{19,22,24,165} our null associations are consistent with a large body of literature suggesting no association between maternal psychosocial stress during pregnancy and preterm birth. For example, a recent systematic review examining the association between depression and preterm birth found that only 25% of studies showed a statistically significant association.¹⁷³ Two additional prospective cohort studies found no association with perceived stress and preterm birth.^{174,175} Additionally, Dole et al. reported that perceived neighborhood safety and social support were not associated with preterm birth.²² Our findings contribute to the growing body of

literature exploring associations between stress and birth outcomes across different populations.

It is possible that we did not observe any associations between psychosocial stress, gestational age, and preterm birth due to our inability to measure psychosocial stress across the life course. In PROTECT, psychosocial stress was measured during pregnancy and focused on self-reported, acute psychosocial stress occurring immediately before (i.e., negative life experiences) and during pregnancy (i.e., neighborhood perceptions, perceived stress, depression, social support). Previous research has suggested that women's reproductive potential is modified based on early life experiences and cumulative allostatic load, the body's chronic accumulation of stress.¹⁶² Thus, the accumulation of psychosocial stress across the life course, rather than psychosocial stress specifically during pregnancy, may be more strongly associated with adverse pregnancy outcomes,¹⁶² and may represent one explanation for our null findings. Importantly, our study was adequately powered to detect an association between psychosocial stress and preterm birth using a OR of 1.5 and 2.0, which gives us confidence that our null results are not due to inadequate sample size.

Discrepancies in our findings may also be due to differences within study populations. Our study population experiences lower levels of other psychosocial stress indicators relative to other populations.^{24,176} For example, in our analytic sample, the mean CES-D score was 11.6 (SD=9.08). In comparison, the mean CES-D score in the Boston Puerto Rico Health Study was 22 (SD=13.5), which is notably higher than what was observed in the PROTECT population. Additionally, the mean CES-D score was

21.8 (SD=7.5) among a convenience sample of women recruited from primary care clinics in San Juan, PR.¹³⁴

Factors associated with low socioeconomic status, such as smoking during pregnancy, young maternal age, being unmarried, and low educational attainment, are also frequently cited as risk factors for preterm birth^{19,24} and may represent another explanation for our null results. Notably, women enrolled in the PROTECT study population are relatively higher socioeconomic status compared to other populations. For example, among women who delivered preterm in our study, a small portion had less than a high school education (13%) and the majority were either married (49%) or living with a partner (32%). In comparison, among the pregnant Puerto Rican cohort in Massachusetts where a statistically significant association between perceived stress and preterm birth was observed, 50% of women who delivered preterm had less than a high school education and 54% were married or living with a partner.²⁴

Our findings should be interpreted in light of some limitations. First, it is difficult to quantify psychosocial stress. However, we included five different measures of psychosocial stress and it is important to note that all psychosocial stress scales used in this analysis have been used in other populations. An additional limitation is the timing of our psychosocial stress measures, which were administered at the 2nd and 3rd study visits. No measure of psychosocial stress prior to pregnancy, which may better measure stress across the life course, was available in PROTECT. Lastly, we did not include biomarkers of stress, such as cortisol or oxidative stress, in this analysis. Previous research has suggested that biomarkers may not correlate with an individual's sense of well-being,⁴⁸ which may be more representative of stress across the life course.

Despite these limitations, our study has many strengths. An advantage of the PROTECT cohort is the prospective study design. Psychosocial stress measures were collected during the 2nd and 3rd trimester and therefore prior to birth outcomes. A wide range of covariates were also available in PROTECT and we found little evidence of confounding by covariates, as shown by our adjusted estimates changing minimally from the crude. Additionally, many different indices of psychosocial stress were available in this population. This allowed us to explore associations between different parameterizations of psychosocial stress in relation to gestational age and preterm birth. Lastly, our results provide important baseline information of the relationship between psychosocial stress and preterm birth among women in Puerto Rico prior to arrival of Hurricane Maria in September 2017.

Conclusions

To the best of our knowledge, this is the first study examining psychosocial stress as a risk factor for preterm birth among Puerto Ricans residing on the island. In our study population, no indices of psychosocial stress were associated with gestational age or preterm birth. Our results suggest that psychosocial stress is not a major contributor to preterm birth in the PROTECT study population. However, certain demographic characteristics, including those indicative of low socioeconomic status, were commonly seen among women delivering preterm. Future research should explore other environmental factors that may be associated with an increased risk of preterm birth in this population.

Acknowledgements

This work was supported by the NIH National Institute of Environmental Health Sciences grants P42ES017198 and P50ES026049 and the National Institutes of Health Office of the Director grants UG3OD023251 and UH3OD023251 and award U54 MD007600 from the National Institute on Minority Health and Health Disparities at NIH.

Table 6.1. Distributions of demographic characteristics and psychosocial stress parameters by preterm birth status among participants of the PROTECT birth cohort (N=1,047).

Characteristic	Preterm Birth (N=107)	Full Term Birth (N=940)	p-value
	N (%)	N (%)	
Maternal Age, years			0.86
18-24	41 (38.3)	356 (37.9)	
25-29	30 (28.0)	290 (30.9)	
30-34	25 (23.4)	189 (20.1)	
≥35	11 (10.3)	105 (11.1)	
Maternal Education			0.04
<High school	13 (12.2)	65 (6.95)	
High school or equivalent	18 (16.9)	116 (12.4)	
Some college or technical school	41 (38.0)	339 (36.1)	
≥College degree	35 (32.8)	419 (44.6)	
Employment Status			0.03
Unemployed	51 (47.8)	345 (36.7)	
Employed	56 (52.2)	595 (63.3)	
Marital Status			0.05
Single	20 (18.9)	192 (20.4)	
Married	52 (48.2)	538 (57.3)	
Living together	35 (32.9)	209 (22.3)	
Alcohol Use			0.12
Never	64 (59.8)	469 (49.9)	
Before pregnancy	35 (32.6)	416 (44.2)	
Currently drinking	8 (7.57)	55 (5.85)	
Smoking			0.49
Never	95 (88.7)	787 (83.7)	
Before pregnancy	10 (9.35)	123 (13.1)	
Current	2 (1.96)	30 (3.22)	
Insurance Status			<0.01
Private	51 (47.5)	603 (64.1)	
Public	54 (50.2)	320 (34.0)	
Unemployed	2 (2.34)	17 (1.83)	
Social Support			0.32
High	38 (35.3)	371 (39.5)	
Medium	38 (35.5)	299 (31.9)	
Low	31 (29.2)	269 (28.7)	
Perceived Stress			0.40
Low	39 (36.7)	318 (33.8)	
Medium	39 (36.8)	325 (34.6)	
High	28 (26.4)	297 (31.6)	
Negative Life Experiences			0.22
Low	35 (32.7)	342 (36.4)	
Medium	41 (38.3)	312 (33.1)	

	High	31 (29.0)	286 (30.5)	
Depression				0.76
	Low	34 (31.7)	327 (34.8)	
	Medium	40 (37.0)	308 (32.7)	
	High	33 (31.3)	305 (32.5)	
Neighborhood Perceptions				0.65
	Low	70 (65.9)	590 (62.8)	
	Medium	27 (25.2)	242 (25.8)	
	High	10 (8.88)	108 (11.4)	

Abbreviations: SD, standard deviation.

Note: p-values calculated from chi-square tests.

Table 6.2. Crude and adjusted¹ change in gestational age (weeks) and 95% confidence intervals in association with psychosocial stress levels in PROTECT (N=1,047).

		Crude β (95% CI)	Adjusted ¹ β (95% CI)
Social Support			
	High	Ref	Ref
	Medium	-0.27 (-0.64, 0.10)	-0.06 (-0.50, 0.37)
	Low	-0.14 (-0.51, 0.24)	0.15 (-0.28, 0.58)
	p trend	0.41	0.47
Perceived Stress			
	Low	Ref	Ref
	Medium	-0.02 (-0.39, 0.36)	0.01 (-0.36, 0.39)
	High	0.10 (-0.23, 0.43)	0.21 (-0.12, 0.55)
	p trend	0.56	0.22
Negative Life Experiences			
	Low	Ref	Ref
	Medium	-0.14 (-0.50, 0.21)	-0.17 (-0.53, 0.18)
	High	-0.03 (-0.37, 0.31)	-0.04 (-0.37, 0.30)
	p trend	0.82	0.79
Depression			
	Low	Ref	Ref
	Medium	-0.14 (-0.47, 0.20)	-0.14 (-0.47, 0.19)
	High	0.01 (-0.33, 0.34)	0.13 (-0.22, 0.48)
	p trend	0.97	0.48
Neighborhood Perceptions			
	Low	Ref	Ref
	Medium	-0.04 (-0.35, 0.27)	-0.06 (-0.36, 0.25)
	High	-0.05 (-0.49, 0.39)	0.01 (-0.43, 0.44)
	p trend	0.76	0.88

Abbreviations: CI, confidence interval; Ref, reference.

¹Models adjusted for maternal age, education, and marital status.

Table 6.3. Crude and adjusted¹ odds ratios of preterm birth and 95% confidence intervals in association with psychosocial stress levels in PROTECT (N=1,047).

		Crude OR (95% CI)	Adjusted ¹ OR (95% CI)
Social Support			
	High	Ref	Ref
	Medium	1.25 (0.72, 2.15)	1.03 (0.55, 1.95)
	Low	1.14 (0.66, 1.97)	0.87 (0.46, 1.63)
	p trend	0.61	0.63
Perceived Stress			
	Low	Ref	Ref
	Medium	0.98 (0.57, 1.68)	0.96 (0.55, 1.67)
	High	0.77 (0.45, 1.32)	0.71 (0.40, 1.23)
	p trend	0.35	0.23
Negative Life Experiences			
	Low	Ref	Ref
	Medium	1.29 (0.76, 2.18)	1.36 (0.79, 2.32)
	High	1.06 (0.62, 1.81)	1.11 (0.64, 1.93)
	p trend	0.80	0.66
Depression			
	Low	Ref	Ref
	Medium	1.24 (0.75, 2.04)	1.29 (0.77, 2.14)
	High	1.06 (0.63, 1.78)	0.98 (0.57, 1.69)
	p trend	0.82	0.96
Neighborhood Perceptions			
	Low	Ref	Ref
	Medium	0.93 (0.58, 1.51)	0.95 (0.58, 1.55)
	High	0.74 (0.35, 1.54)	0.70 (0.33, 1.49)
	p trend	0.43	0.40

Abbreviations: OR, odds ratio; CI, confidence interval; Ref, reference.

¹Models adjusted for maternal age, education, and marital status.

Table 6.4. Adjusted¹ odds ratios of preterm birth and 95% confidence intervals in association with psychosocial stress levels, stratified by level of social support (N=1,047).

		Low Social Support (N=302)	Medium/High Social Support (N=745)	p-interaction
		OR (95% CI)	OR (95% CI)	
Perceived Stress				0.54
	Low	Ref	Ref	
	Medium	0.66 (0.21, 2.06)	1.07 (0.58, 2.00)	
	High	0.70 (0.25, 1.93)	0.64 (0.31, 1.32)	
	p trend	0.54	0.30	
Negative Life Experiences				0.28
	Low	Ref	Ref	
	Medium	1.58 (0.55, 4.59)	1.29 (0.67, 2.49)	
	High	1.44 (0.52, 4.01)	0.89 (0.45, 1.76)	
	p trend	0.52	0.84	
Depression				0.47
	Low	Ref	Ref	
	Medium	1.46 (0.52, 4.15)	1.20 (0.66, 2.2)	
	High	0.70 (0.23, 2.12)	1.16 (0.61, 2.24)	
	p trend	0.37	0.62	
Neighborhood Perceptions				0.90
	Low	Ref	Ref	
	Medium	0.93 (0.38, 2.28)	0.93 (0.51, 1.72)	
	High	0.54 (0.15, 1.99)	0.80 (0.30, 2.14)	
	p trend	0.39	0.66	

Abbreviations: OR, odds ratio; CI, confidence interval; Ref, reference.

¹Models adjusted for maternal age, education, and marital status.

Note: p-values for interaction were calculated using ANOVA.

CHAPTER 7

CONCLUSIONS

Summary

This dissertation addressed the public health issue of psychosocial stress during pregnancy in Puerto Rico. Psychosocial stress stems from perceptions of threats which result in discomfort, emotional tension, and difficulty adjusting. During pregnancy, psychosocial stress has been associated with a number of adverse pregnancy outcomes, including preterm birth, and previously has not been explored in Puerto Rico. The rates of preterm birth in Puerto Rico are some of the highest both globally and in the United States. Our study used data from a subset of women enrolled in an ongoing, prospective cohort study, Puerto Rico Testsite for Exploring Contamination Threats (PROTECT), from 2011 to 2017. The PROTECT cohort was established as part of the Superfund Research Program and women are recruited from Puerto Rico's Northern Karst region. The Northern Karst Region of Puerto Rico is home to the majority of the superfund sites on the island. Here, the superfund sites over the Karst aquifers, and water sampling in this region has consistently shown the presence of many environmental contaminants.

This dissertation discussed three major areas of interest. In the first aim, we sought to describe psychosocial stress in the PROTECT study population and to explore the pathways through which psychosocial stress indices influenced one another. Second, aim 2 investigated oxidative stress as a potential physiologic response

to psychosocial stress during pregnancy, hypothesizing that women in the highest compared to lowest tertile of psychosocial stress would have increased biomarkers of oxidative stress. Lastly, aim 3 examined psychosocial stress as a potentially modifiable risk factor for preterm birth, hypothesizing that women in the highest tertile of psychosocial stress would be more likely to deliver preterm compared to women low stress women. Although our results from aims 2 and 3 did not support our hypotheses, our results provide important baseline information regarding psychosocial stress during pregnancy in Puerto Rico prior to the arrival of Hurricane Maria in September 2017.

Strengths and Limitations

Our results should be interpreted in light of its strengths and limitations. First, our study included 5 different measures of psychosocial stress, which allowed us to explore many different indices of stress that may be relevant during pregnancy. Nonetheless, it is difficult to quantify psychosocial stress during pregnancy and cut points for determining high and low stress vary widely across studies. However, the questionnaires used to measure psychosocial stress in the PROTECT study have been validated and used in other studies. Among psychosocial stress measures included in this dissertation, only the CES-D is a clinical screening tool and PROTECT did not include diagnostic mental health measures, which are the gold standard for determining mental health. Additionally, some psychosocial stress measures were administered at the same study visit. Thus, temporality is a concern in aim 1, as we are unable to determine causality between psychosocial stress measures. However, all associations we observed in our final model have been observed in the literature, giving us confidence in our results. In the second aim, we included 3 biomarkers of oxidative

stress. 8-isoprostane is thought to be one of the best biomarkers of oxidative stress because it is stable, including during human pregnancy, unaffected by lipids in the diet, and is detectable in urine. Additionally, we also included 8-isoprostane's major metabolite, which is hypothesized to be a more sensitive biomarker in urine. In aim 3, psychosocial stress was measured prior to birth outcomes. Lastly, PROTECT employs a prospective cohort study design, which is a methodological advancement over other retrospective and cross-sectional studies.

Suggestions for Future Research

The findings from this dissertation have many implications for future research. The associations between psychosocial stress measures that were observed in aim 1 should be explored in other populations, such as Puerto Ricans in the mainland United States who may be different from women enrolled in PROTECT. Other domains of psychosocial stress, such as anxiety and resiliency, are potentially important in this context and further research is needed to see how these factors may contribute to increased psychosocial stress and ultimately preterm birth. Although we observed no associations between psychosocial stress and oxidative stress, other biomarkers of stress, such as cortisol and telomere length may be associated with psychosocial stress during pregnancy and warrant further exploration. It is also important to explore associations between oxidative stress and preterm birth, as these associations have been observed in other studies. In addition, other environmental factors, such as phthalates and air pollution, should be explored in relation to oxidative stress as they may be contributing to Puerto Rico's high preterm birth rate. Previous research in the PROTECT cohort has shown that environmental factors increase oxidative stress

levels.¹⁷⁷ Oxidative stress is likely a mediating pathway linking environmental exposures to preterm birth.³⁴ Lastly, it was important to examine the associations between hurricane-specific psychosocial stress and preterm birth after the arrival of Hurricane Maria, as psychosocial stress during pregnancy may have changed as a result of the hurricane.

Conclusions

In our first aim, we found that women who of lower socioeconomic status, including women who are between ages 18-24, unemployed, and have public insurance, have increased psychosocial stress compared to reference groups. We also found evidence that women with high perceived stress also have high levels of depression. In comparison to other studies of pregnant women, the PROTECT study population is a low stress cohort. Despite PROTECT women experiencing less psychosocial stress, associations between psychosocial stress measures that were observed in other studies were also observed here.

In our second and third aims, psychosocial stress was not associated with gestational age or preterm birth and we observed no associations between psychosocial stress and oxidative stress biomarkers. Although we did not observe any statistically significant associations, our results from aim 2 suggest that women who experience high and medium depression compared to low may have increased oxidative stress biomarkers in the direction expected. This is consistent with some of our prior work in another pregnancy cohort suggesting that women who experience depression have increased 8-isoprostane and this finding should be explored in other studies. It is possible that we observed no associations between psychosocial stress,

oxidative stress, and preterm birth due to the low levels of psychosocial stress in this population and it is possible that the effects of psychosocial stress on preterm birth are only observed when psychosocial stress levels are sufficiently high. Our null results may also be explained by the low levels of psychosocial stress observed among PROTECT participants.

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