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Repeated Measures of Urinary Oxidative Stress Biomarkers and Preterm Birth in Puerto Rico

Stephanie M. Eick, PhD, MPH[1], **Kelly K. Ferguson, PhD**[2,3], **Ginger L. Milne, PhD**[4], **Rafael Rios-McConnell, MS**[5], **Carmen Velez-Vega, PhD, MSW**[5], **Zaira Rosario, MS**[5], **Akram Alshawabkeh, PhD**[6], **José F. Cordero, MD, MPH**[7], **John D. Meeker, ScD**[3]

[1]Program on Reproductive Health and the Environment, Department of Obstetrics, Gynecology and Reproductive Services, University of California San Francisco, San Francisco, CA, United States

[2]Epidemiology Branch, National Institute of Environmental Health Sciences, Research Triangle Park, NC, United States

[3]Department of Environmental Health Sciences, University of Michigan School of Public Health, Ann Arbor, MI, United States

[4]Division of Clinical Pharmacology, Vanderbilt University Medical Center, Nashville, TN, United States

[5]University of Puerto Rico Graduate School of Public Health, UPR Medical Sciences Campus, San Juan, PR, United States

[6]College of Engineering, Northeastern University, Boston, MA, United States

[7]Department of Epidemiology and Biostatistics, College of Public Health, University of Georgia, Athens, GA, United States

Abstract

Background: Preterm birth (PTB; gestational age <37 weeks), the leading cause of infant morbidity and mortality worldwide, is of particular concern in Puerto Rico. Rates of PTB in Puerto Rico peaked at 20% in 2006, which are historically some of the highest in the world. Oxidative stress and inflammation have been implicated as contributors to adverse birth outcomes, including PTB, and these associations have not been explored in Puerto Rico. Our objective was to examine associations between urinary oxidative stress biomarkers and PTB in the Puerto Rico Testsite for Exploring Contamination Threats (PROTECT) pregnancy cohort (N=469).

Correspondence: John D. Meeker, ScD, University of Michigan School of Public Health, Department of Environmental Health Sciences, 1835 SPH I, 1415 Washington Heights, Ann Arbor, MI 48109-2029, Phone: 1-734-764-7184, Fax: 1-734-936-7283, meekerj@umich.edu.

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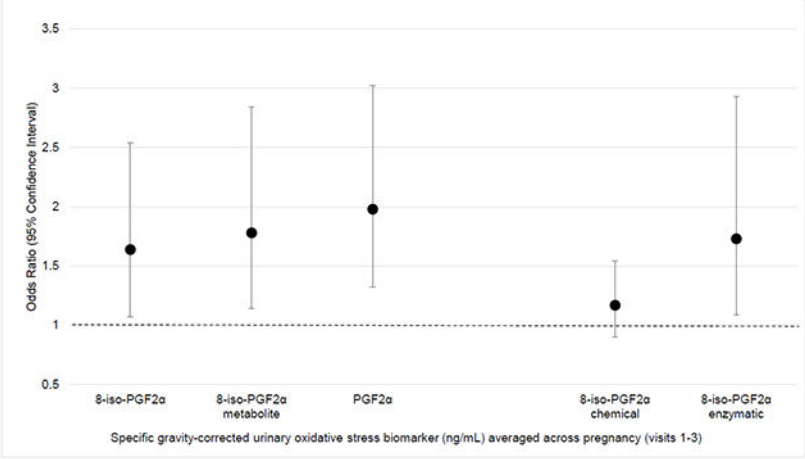
Methods: 8-iso-prostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$), its primary metabolite, and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) were included as biomarkers of oxidative stress or inflammation. Biomarkers were measured in urine samples collected at up to 3 timepoints across pregnancy (mean 18, 24, 28 weeks gestation). We quantified the proportion of 8-iso-$PGF_{2\alpha}$ originating from oxidative stress and inflammation pathways with a formula based on the ratio of 8-iso-$PGF_{2\alpha}$ to $PGF_{2\alpha}$. Logistic regression models were used to calculate adjusted odds ratios (OR) for associations between average biomarker concentrations from each woman (visits 1-3) and PTB. Associations between biomarker concentrations at each study visit and PTB were analyzed in separate models.

Results: Averaged levels of 8-iso-$PGF_{2\alpha}$, its primary metabolite, and $PGF_{2\alpha}$ were associated with increased odds of PTB (OR=1.64, 95% confidence interval [CI]=1.07-2.54; OR=1.79, 95% CI=1.14-2.84; OR=1.98, 95% CI=1.32-3.02, respectively). Odds ratios for PTB were greater in magnitude in association with oxidative stress biomarkers measured later in pregnancy. The fraction of 8-iso-$PGF_{2\alpha}$ derived from inflammation was associated with PTB (OR=1.73, 95% CI=1.09, 2.93), while the fraction of 8-iso-$PGF_{2\alpha}$ derived from oxidative stress was not associated with PTB (OR=1.17, 95% CI=0.90, 1.54).

Conclusions: Our results suggest that oxidative stress and inflammation, as measured by these biomarkers, may be important contributors to PTB. Further research is needed to improve our understanding of the role these biomarkers may play in the causal pathway between environmental factors and PTB.

Graphical Abstract



Keywords

oxidative stress; preterm birth; isoprostanes; repeated measures; Puerto Rico; epidemiology

Introduction

Preterm birth (PTB), defined as delivery prior to 37 weeks gestation, is one of the leading causes of infant morbidity and mortality worldwide.[1] Each year, roughly 15 million babies are born preterm and an estimated 35% of neonatal deaths are due to causes directly related to PTB.[1] PTB is particularly problematic in Puerto Rico, where the rates of PTB are some of

the highest in the U.S. and the world. Puerto Rico's PTB rate was 19.9% in 2006[2] and although this rate has since declined to 11.4% in 2017, it remains high relative to the continental U.S.[3]

The biologic mechanisms leading to PTB are incompletely understood. Oxidative stress may represent one possible mechanism. Oxidative stress biomarkers have been associated with adverse pregnancy outcomes[4,5] and several studies have shown that oxidative stress biomarkers are increased among women who go on to experience PTB or shortened gestation.[6–8] However, the strength of association remains inconsistent across studies and discrepancies may in part be due to differences in biomarkers used to measure oxidative stress.

In the present study, we sought to examine associations between multiple biomarkers of oxidative stress and PTB in the Puerto Rico Testsite for Exploring Contamination Threats (PROTECT) pregnancy cohort. 8-isoprostane-prostaglandin-$F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$), the major 8-isoprostane-$PGF_{2\alpha}$ metabolite, and prostaglandin-$F_{2\alpha}$ ($PGF_{2\alpha}$) were included as biomarkers of oxidative stress. 8-iso-$PGF_{2\alpha}$ is widely studied with respect to pregnancy outcomes and is considered one of the best biomarkers of oxidative stress because of its reliability and stability during human pregnancy.[9,10] However, the 8-iso-$PGF_{2\alpha}$ metabolite may be a more sensitive and superior biomarker of oxidative stress than 8-iso-$PGF_{2\alpha}$[11] and suggestive associations between the metabolite and spontaneous PTB have been observed.[12] Lastly, we included $PGF_{2\alpha}$ which is less studied within the context of human pregnancy but has been previously linked to the inflammation mechanisms underlying PTB.[13] Previously, in the PROTECT study population, we found that indicators of lower socioeconomic status were associated with elevated levels of these biomarkers.[14] Here, we build upon this work and examined longitudinal associations with these biomarkers and PTB, hypothesizing that women with increased oxidative stress biomarkers would be more likely to deliver preterm.

Materials and Methods

Study Population

Women included in the current analysis are a subset of women enrolled in the PROTECT cohort. PROTECT is an ongoing, prospective cohort study in Northern Puerto Rico and recruitment methods have been previously described.[15,16] The 469 women included in this analysis delivered between 2011 and 2017 and were recruited between 14 and 20 weeks gestation from affiliated hospitals and prenatal clinics. Exclusion criteria for PROTECT included: maternal age less than 18 or greater than 40 years, maternal residence was outside of the Northern Karst aquifer region, use of oral contraceptives for 3 months prior to conception, and preexisting obstetric and medical complications (e.g., diabetes). All participants are invited to complete up to 3 study visits targeted at 16-20 weeks gestation, 20-24 weeks gestation, and 24-28 weeks gestation. Demographic information was collected by questionnaire at the first visit and spot urine samples were collected at all study visits. The study was approved by the Institutional Review Boards at the University of Puerto Rico, Northeastern University, University of Michigan, and University of Georgia. All women provided written, informed consent prior to participating in PROTECT.

Oxidative Stress Biomarker Assessment

Urine samples were collected in polypropylene containers, divided into aliquots, and frozen at −80 °C until analysis.[17] Free 8-iso-$PGF_{2\alpha}$, its major metabolite 2,3-dinor-5,6-dihydro-15-F_{2t}-isoprostane, and $PGF_{2\alpha}$ were analyzed by the Eicosanoid Core Laboratory at Vanderbilt University Medical Center (Nashville, TN) as biomarkers of oxidative stress or inflammation. This was done using stable isotype dilution gas chromatography-negative ion chemical ionization-mass spectrometry in samples for 469 participants (N= 270 at visit 1, N= 349 at visit 2, N= 217 at visit 3). This method requires a C18 Sep-Pak 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 prior to extraction. Further details describing measurement of oxidative stress biomarker concentrations are available elsewhere.[18]

8-iso-$PGF_{2\alpha}$ is generated through both chemical free radical oxidation and enzymatic lipid peroxidation pathways. Thus, 8-iso-$PGF_{2\alpha}$ may not be solely a biomarker of oxidative stress. To account for these separate mechanisms, we additionally examined the proportion of 8-iso-$PGF_{2\alpha}$ that was derived from chemical free radical oxidation and enzymatic lipid peroxidation pathways.[19] This was done utilizing the ratio of 8-iso-$PGF_{2\alpha}$ to $PGF_{2\alpha}$ which has been previously described in detail by van ’t Erve et al. and was calculated using a custom interface for the R package “Constrained Linear Mixed Effects (CLME)”.[19] The chemical fraction captures non-enzymatic lipid peroxidation, and the enzymatic fraction is generated from prostaglandin-endoperoxide synthases and is more reflective of inflammation. As a sensitivity analysis, associations between PTB and the chemical and enzymatic fractions of 8-iso-$PGF_{2\alpha}$ were analyzed separately to determine which pathway drove the associations between overall 8-iso-$PGF_{2\alpha}$ and PTB.

To account for urine dilution, urinary specific gravity (SpG) was measured using a digital handheld refractometer. All urinary oxidative stress biomarker 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.[17,20] Ox is the measured oxidative stress concentration and Ox_c is the SpG-corrected measure. All SpG-corrected oxidative stress biomarker concentrations were natural log transformed for normality.

Gestational Age

Gestational age was assessed based on American College of Obstetricians and Gynecologists guidelines using a combination of self-reported date of last menstrual period collected at the first study visit and first ultrasound estimates of gestational age.[16,21] For analysis, we categorized gestational age into PTB (<37 weeks gestation) and full term birth (≥37 weeks gestation). PTB was classified as spontaneous if premature rupture of the membranes, spontaneous preterm labor, or both was present.[22]

Statistical Analysis

All statistical analyses were conducted in R Version 3.5.0 and SAS 9.4 (Cary, NC). The distributions of measured urinary oxidative stress biomarker concentrations were examined using geometric means, geometric standard deviations, and selected percentiles. Intraclass

correlation coefficients (ICC) measurements from all study visits were calculated as an additional measure of individual variability in biomarker concentrations.[23] ICC values range from 0 to 1 where ICC values of 1 indicate perfect reproducibility, values closer to 0 indicate greater variability, and values between 0.40 and 0.75 indicate good reliability.[23]

Geometric average biomarker concentrations were obtained by taking the geometric mean of the available SpG-corrected oxidative stress biomarker concentrations from all participants at visits 1-3. For example, if a participant had 8-iso-$PGF_{2\alpha}$ measured at each visit, we took the geometric mean of SpG-corrected 8-iso-$PGF_{2\alpha}$ at visits 1-3. If a participant had SpG-corrected 8-iso-$PGF_{2\alpha}$ at visit 1 only, we used only that measure. No participants were missing oxidative stress biomarkers at visit 3 as a result of delivering preterm prior to the visit.

Logistic regression was used to calculate crude and adjusted odds ratios (OR) and 95% confidence intervals (CI) for the associations between averaged oxidative stress biomarkers and PTB. Maternal age, maternal education, employment status, marital status, alcohol use, smoking status, and insurance status were examined as potential covariates in adjusted models. Covariates retained in adjusted models were associated with oxidative stress biomarkers in bivariate analyses and have been previously associated with PTB. We additionally examined associations between PTB and SpG-corrected urinary oxidative stress biomarkers at each visit using individual logistic regression models. This was done to determine whether oxidative stress biomarkers at a particular timepoint were differentially associated with PTB. Gestational age at study visit and covariates from averaged models were retained in adjusted individual models. For consistency with previous research and to increase interpretability, ORs for PTB were standardized to reflect odds associated with an interquartile range (IQR) increase in SpG-corrected oxidative stress markers.[12,24]

Results

A detailed description of the demographics in the overall PROTECT cohort is available elsewhere.[16] There were 469 women included in the present analysis who had urine samples analyzed for oxidative stress biomarkers. Within our analytic sample, 50 participants delivered preterm (11.2%), of this group 30 were classified as having a spontaneous PTB. The largest percentage of women included in the current analysis were between 18-24 years of age (40%), had a college degree or higher (41%), were employed (62%), and were married (54%) (Table 1). At the first study visit, few women reported ever smoking (4%) or consuming alcohol (5%) (Table 1). Demographic characteristics of participants included in our analytic sample were similar to that of the larger PROTECT cohort.[16]

Concentrations of 8-iso-$PGF_{2\alpha}$ were higher among women who were between 18-24 compared to 25-29 years of age and who had public compared to private insurance (p-value <0.01).[14] The geometric mean of the 8-iso-$PGF_{2\alpha}$ metabolite was higher among women with a high school education or equivalent compared to women with a college education or greater and among women who were unemployed compared to employed (p-value <0.01).[14] Associations between demographic characteristics and $PGF_{2\alpha}$ were similar but less precise.

ICC values for 8-iso-$PGF_{2\alpha}$ and the 8-iso-$PGF_{2\alpha}$ metabolite demonstrated good temporal reliability (ICC=0.52, 95% CI=0.47-0.58 for both biomarkers). 8-iso-$PGF_{2\alpha}$ and the 8-iso-$PGF_{2\alpha}$ metabolite were also moderately correlated (Spearman R=0.67, p-value <0.01). ICC values for $PGF_{2\alpha}$ were also moderate or good, but were somewhat less reliable (ICC=0.41, 95% CI=0.35-0.47).

Distributions of oxidative stress biomarkers are shown in Table 2. Levels of 8-iso-$PGF_{2\alpha}$ and the 8-iso-$PGF_{2\alpha}$ metabolite across preterm and term births were similar at study visits 1 and 2 (Figure 1; Figure 2). $PGF_{2\alpha}$ concentrations were elevated relative to those of 8-iso-$PGF_{2\alpha}$ and the metabolite (Table 2). Additionally, $PGF_{2\alpha}$ levels at visit 2 were significantly higher among women who delivered preterm compared to term (Figure 3). For all oxidative stress biomarkers, levels at visit 3 were significantly higher among women who delivered preterm compared to term (Figures 1–3).

Maternal age, marital status, and maternal education were retained as covariates in adjusted logistic regression models. Adjusted ORs of PTB in association with an IQR increase in oxidative stress biomarkers are shown in Table 3. An IQR increase in 8-iso-$PGF_{2\alpha}$ (OR=1.64, 95% CI=1.07-2.54), the 8-iso-$PGF_{2\alpha}$ metabolite (OR=1.79, 95% CI=1.14-2.84), and $PGF_{2\alpha}$ (OR=1.98, 95% CI=1.32-3.02) were all associated with increased odds of PTB in models of pregnancy averages of these markers. Individual logistic regression models stratified by study visit indicated that odds of PTB in relation to oxidative stress biomarkers increased moderately throughout pregnancy. For example, an IQR increase in $PGF_{2\alpha}$ was associated with a 1.24 (95% CI=0.78, 1.96), 1.60 (95% CI=1.01, 2.58), and 2.01 (95% CI=1.03, 4.17) increase in odds of PTB at visits 1, 2, and 3, respectively. Associations between oxidative stress biomarkers and PTB were similar in crude analyses.

In our sensitivity analyses distinguishing between the chemical and enzymatic fractions of 8-iso-$PGF_{2\alpha}$, we observed that the association between averaged 8-iso-$PGF_{2\alpha}$ and PTB appeared to be driven by associations with inflammation (Table 3). An IQR increase in the enzymatic fraction of 8-iso-$PGF_{2\alpha}$ was associated with increased odds of PTB (OR=1.73, 95% CI=1.09, 2.93). The corresponding OR for the fraction of 8-iso-$PGF_{2\alpha}$ derived from oxidative stress was null (OR=1.17, 95% CI=0.90, 1.54).

Discussion

In the present study, we examined the associations between multiple biomarkers of oxidative stress and PTB using data from a prospective pregnancy cohort in Puerto Rico. Findings from our study showed that levels of 8-iso-$PGF_{2\alpha}$, the 8-iso-$PGF_{2\alpha}$ metabolite, and $PGF_{2\alpha}$ are elevated among women who deliver preterm. Additionally, our sensitivity analysis with the chemical and enzymatic fractions of 8-iso-$PGF_{2\alpha}$ revealed that the association between 8-iso-$PGF_{2\alpha}$ and PTB may be attributable to elevated inflammation. In this study, oxidative stress biomarker concentrations measured later in pregnancy were more strongly associated with PTB relative to levels measured earlier in pregnancy. Results from our study contribute to the growing body of literature suggesting that oxidative stress is on the causal pathway to PTB.

The positive association between increasing 8-iso-$PGF_{2\alpha}$ and PTB that we identified is consistent with previous research.[6–8] For example, a case-control study in Boston found that an increase in 8-iso-$PGF_{2\alpha}$ was associated with increased odds of spontaneous PTB and that associations were greater with 8-iso-$PGF_{2\alpha}$ levels measured later in pregnancy.[6] A second study of 237 premature infants showed that median 8-iso-$PGF_{2\alpha}$ in cord blood was higher among infants born at less than 28 weeks gestation compared to those born between 34-36 weeks gestation.[25] Two additional studies have found that levels of 8-iso-$PGF_{2\alpha}$ measured in amniotic fluid were higher among pregnancies complicated by preterm premature rupture of the membranes (pPROM)[26] and term PROM.[27] Higher median levels of plasma 8-iso-$PGF_{2\alpha}$ have also been observed among women who go on to develop preeclampsia and deliver small for gestational age infants,[28] although that study did not observe an association between 8-iso-$PGF_{2\alpha}$ and PTB.

An important strength of our study is that we included multiple biomarkers of oxidative stress. In addition to 8-iso-$PGF_{2\alpha}$, we also measured the 8-iso-$PGF_{2\alpha}$ metabolite. The effect estimates and statistical associations we observed between the 8-iso-$PGF_{2\alpha}$ metabolite and PTB were larger and stronger, both in averaged models and models specific to study visits, compared to associations with 8-iso-$PGF_{2\alpha}$ and PTB. Our positive findings between the metabolite and PTB are confirmed by a recent study showing that an IQR increase in the 8-iso-$PGF_{2\alpha}$ metabolite was associated with a 44% increase in odds of PTB.[12] The 8-iso-$PGF_{2\alpha}$ metabolite is hypothesized to be more sensitive than 8-iso-$PGF_{2\alpha}$ in urine[11] and eicosanoid production is thought to be best assessed by measurement of urinary metabolites,[29,30] which may be one reason why the associations we observed were greater in magnitude compared to 8-iso-$PGF_{2\alpha}$ itself.

It is plausible that oxidative stress may be one mechanism by which environmental exposures impact PTB. Previous research has identified associations between increases in environmental contaminants and increased oxidative stress levels.[10,17] For example, elevated levels of oxidative stress have been observed among pregnant women with elevated concentrations of certain polycyclic aromatic hydrocarbons, phthalates, phenols, and parabens.[24,31,32] Increases in oxidative stress levels have also been observed among individuals who smoke, are obese, and experience psychological stress,[20,33] all of which have been previously associated with an increased risk of PTB.[16,34,35] Smoking, obesity, and psychological stress have the potential to cause redox imbalance, which could lead to an increased consumption of antioxidant defenses and trigger uterine contractions.[33] Inflammatory cytokines, such as interleukin-6 and C-reactive protein, also have been associated with an increased risk of PTB[36] and may represent an additional pathway through which these exposures may influence PTB risk. Future research in this cohort will determine the host of contributing factors to elevated levels of these biomarkers.

Oxidative stress could be contributing to PTB and shortened gestation through multiple pathways. One plausible mechanism is that oxidative stress is acting as a precursor to spontaneous labor.[33] Increased oxidative stress could lead to accelerated senescence of the maternal-fetal membranes, myometrial activation, and cervical ripening.[33] Oxidative damage could also lead to apoptosis and telomere damage, leading to pPROM and

spontaneous PTB.[33] Finally, increased oxidative stress could lead to an increased risk of infection as a result of reduced immune function.[33]

Our sensitivity analyses used a novel approach to differentiate between the chemical and enzymatic fractions of 8-iso-$PGF_{2\alpha}$ that has not been previously explored in this context. Previously, it was thought that 8-iso-$PGF_{2\alpha}$ was only generated through chemical lipid peroxidation. However, more recent studies have shown that 8-iso-$PGF_{2\alpha}$ can also be generated from enzymatic pathways, which capture cyclooxygenase activity and is responsive to inflammation.[19] In this study, we observed that the association between urinary 8-iso-$PGF_{2\alpha}$ and PTB was largely driven by the portion of 8-iso-$PGF_{2\alpha}$ derived from the enzymatic pathway. Thus, the relationship between 8-iso-$PGF_{2\alpha}$ and PTB we identified may be a result of increases in inflammation. Inflammation may be leading to PTB through increased inflammatory cytokines, which are associated with decreased membrane structural integration, myometrial activation, and cervical ripening.[33] We also observed a strong association between urinary $PGF_{2\alpha}$ and PTB. Proinflammatory cytokines induce prostaglandin production, which promotes cervical ripening and $PGF_{2\alpha}$ to stimulate uterine contractions.[13] Prostaglandins are generally metabolized in less than five minutes, thus urinary prostaglandins levels are more indicative of systemic production than plasma levels. Overall, our results provide strong evidence that the associations we observed between biomarkers of oxidative stress and PTB may in part be due to the upregulation of inflammation pathways.

Our study is not without limitations, as we did not have a large enough sample size to examine subtypes of PTB. Previous literature has shown that increased 8-iso-$PGF_{2\alpha}$ is more strongly linked to PTB with spontaneous presentation.[6] Despite our limitations, our study has many strengths. First, PROTECT employs a prospective cohort design and we had multiple measures of oxidative stress throughout pregnancy. This allowed us to create stable, average measures of oxidative stress, which may be crucial since we observed that oxidative stress levels were somewhat variable over gestation in this study population. In addition, these repeated measures allowed us to examine associations between oxidative stress levels and PTB at different timepoints. Susceptibility to oxidative stress may vary during different exposure windows, which is supported by the findings presented here. Second, we included multiple biomarkers of oxidative stress, which allowed us to examine many biologically relevant associations. These biomarkers were also measured using a highly sensitive and specific mass spectrometry method as opposed to immunoassay-based methods commonly used in other studies. Third, isoprostanes are some of the best biomarkers of oxidative stress because they are reliable, unaffected by lipids in the diet, and stable, including during human pregnancy.[9] Lastly, biomarkers used in this study were measured in urine, which is thought to be better than plasma, as they are not susceptible to autooxidation during storage.[37]

Conclusions

In our study, increased 8-iso-$PGF_{2\alpha}$, the primary 8-iso-$PGF_{2\alpha}$ metabolite, and $PGF_{2\alpha}$ were associated with increased odds of PTB. Furthermore, our additional integration of the 8-iso-$PGF_{2\alpha}$ and PTB relationship revealed that these associations may be more reflective of increased inflammation. Results from our study are consistent with previous research

showing that oxidative stress is associated with adverse pregnancy outcomes. Future research should focus on the role of oxidative stress in inflammation pathways and is needed to better understand the role oxidative stress may play on the causal pathway between environmental factors and PTB.

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Abbreviations:

PTB	preterm birth
PROTECT	Puerto Rico Testsite for Exploring Contamination Threats
OR	odds ratio
CI	confidence interval
8-iso-PGF$_{2\alpha}$	8-isoprostane-prostaglandin-F$_{2\alpha}$
PGF$_{2\alpha}$	prostaglandin-F$_{2\alpha}$
LOD	limit of detection
CLME	Constrained Linear Mixed Effects
SpG	specific gravity
ICC	intraclass correlation coefficient
IQR	interquartile range
pPROM	preterm premature rupture of the membranes

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Highlights:

- 8-iso-$PGF_{2\alpha}$, the 8-iso-$PGF_{2\alpha}$ metabolite, and $PGF_{2\alpha}$ were associated with preterm birth
- Associations between 8-iso-$PGF_{2\alpha}$ and preterm birth may be attributable to inflammation
- Biomarkers measured later in pregnancy were strongly associated with preterm birth than those measured earlier

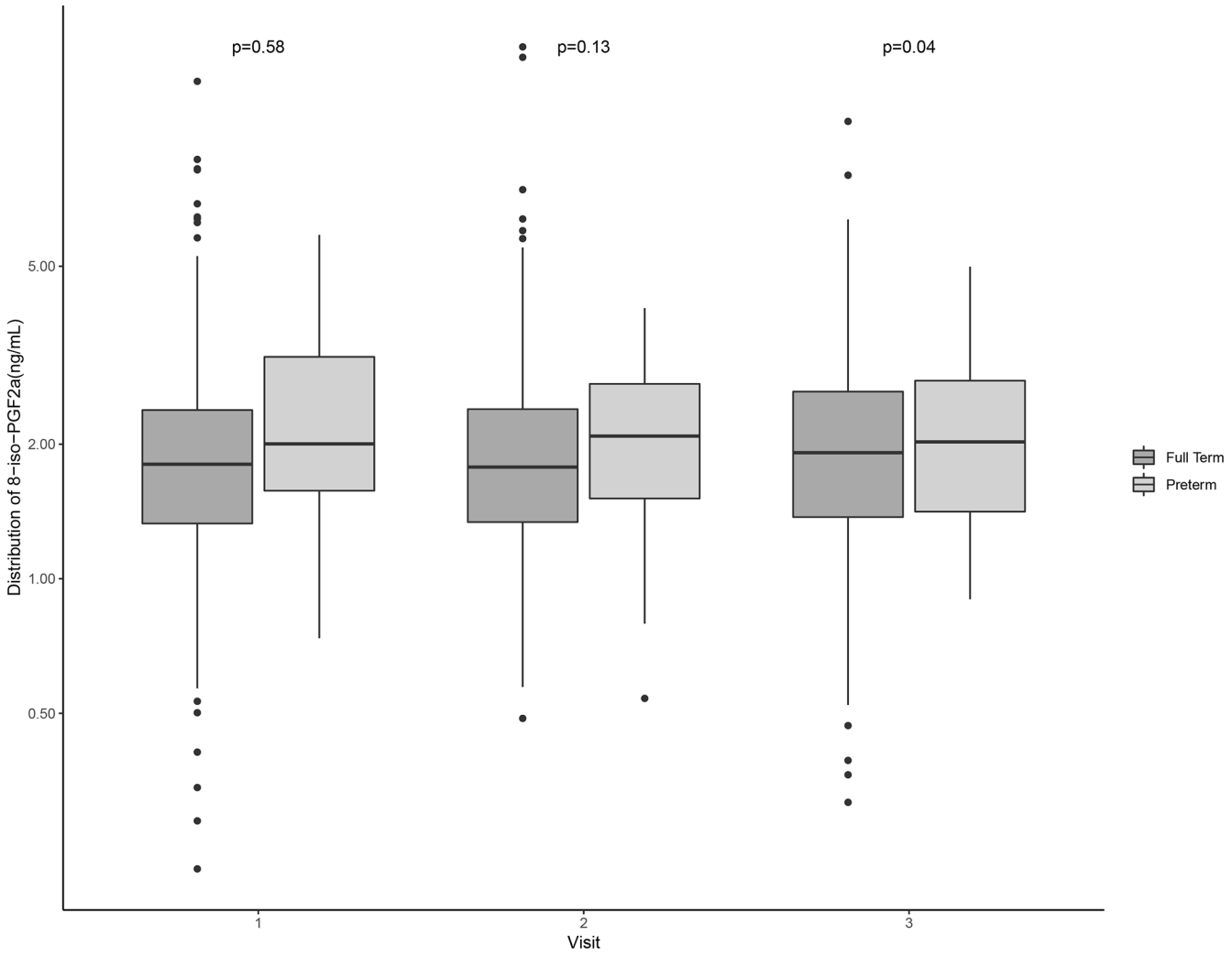

Figure 1.

Distribution of specific gravity-corrected urinary levels of 8-iso-$PGF_{2\alpha}$ (ng/mL) at specific study visits stratified by preterm (gestational age <37 weeks) and full term birth (gestational age ≥37 weeks).

Note: The mean gestational age at study visit was 18, 24, and 28 weeks gestation, respectively. P-values were calculated using t-tests and $p<0.05$ indicates statistical significance.

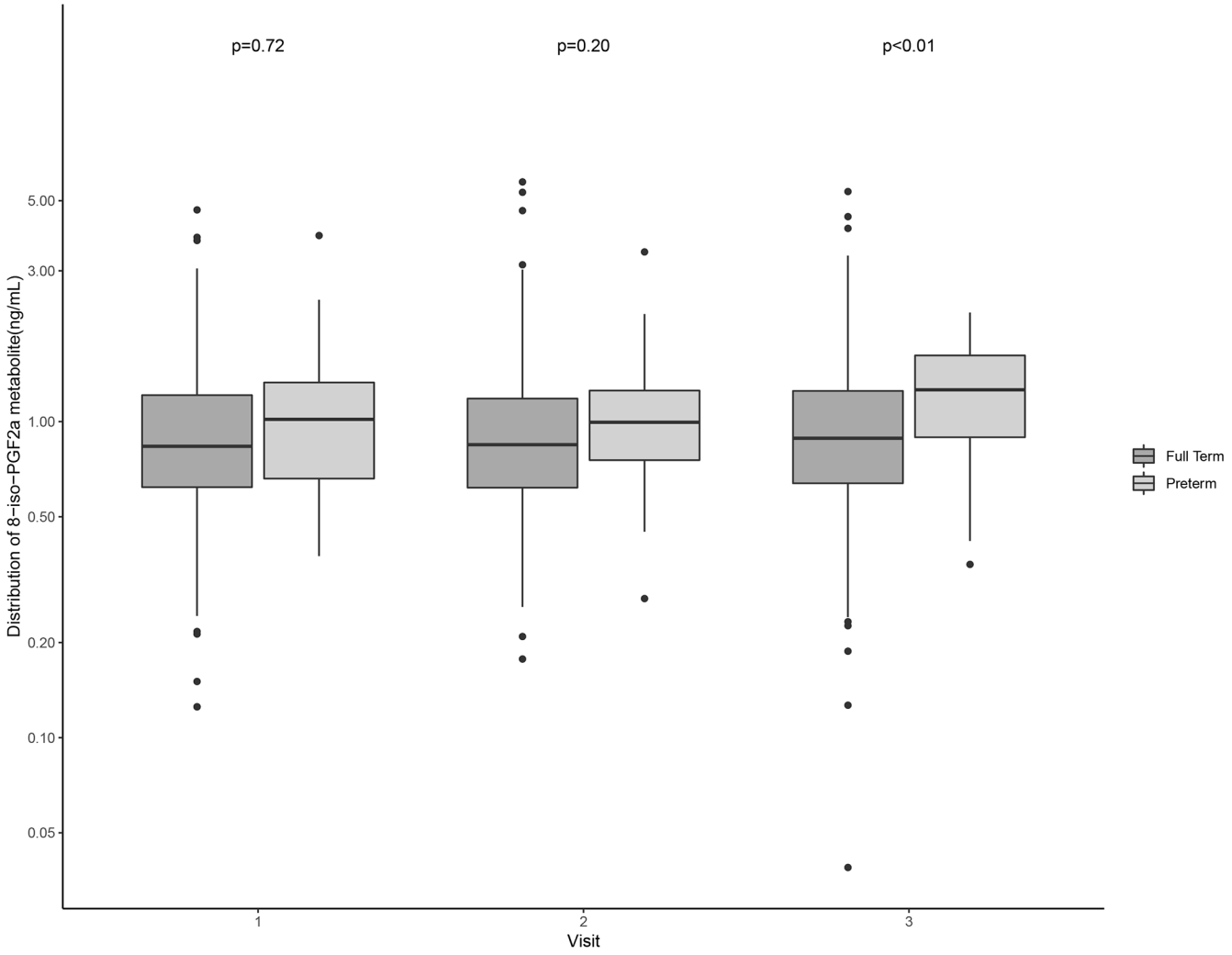

Figure 2.

Distribution of specific gravity-corrected urinary levels of the 8-iso-$PGF_{2\alpha}$ metabolite (ng/mL) at specific study visits stratified by preterm (gestational age <37 weeks) and full term birth (gestational age ≥37 weeks).

Note: The mean gestational age at study visit was 18, 24, and 28 weeks gestation, respectively. P-values were calculated using t-tests and p≥0.05 indicates statistical significance.

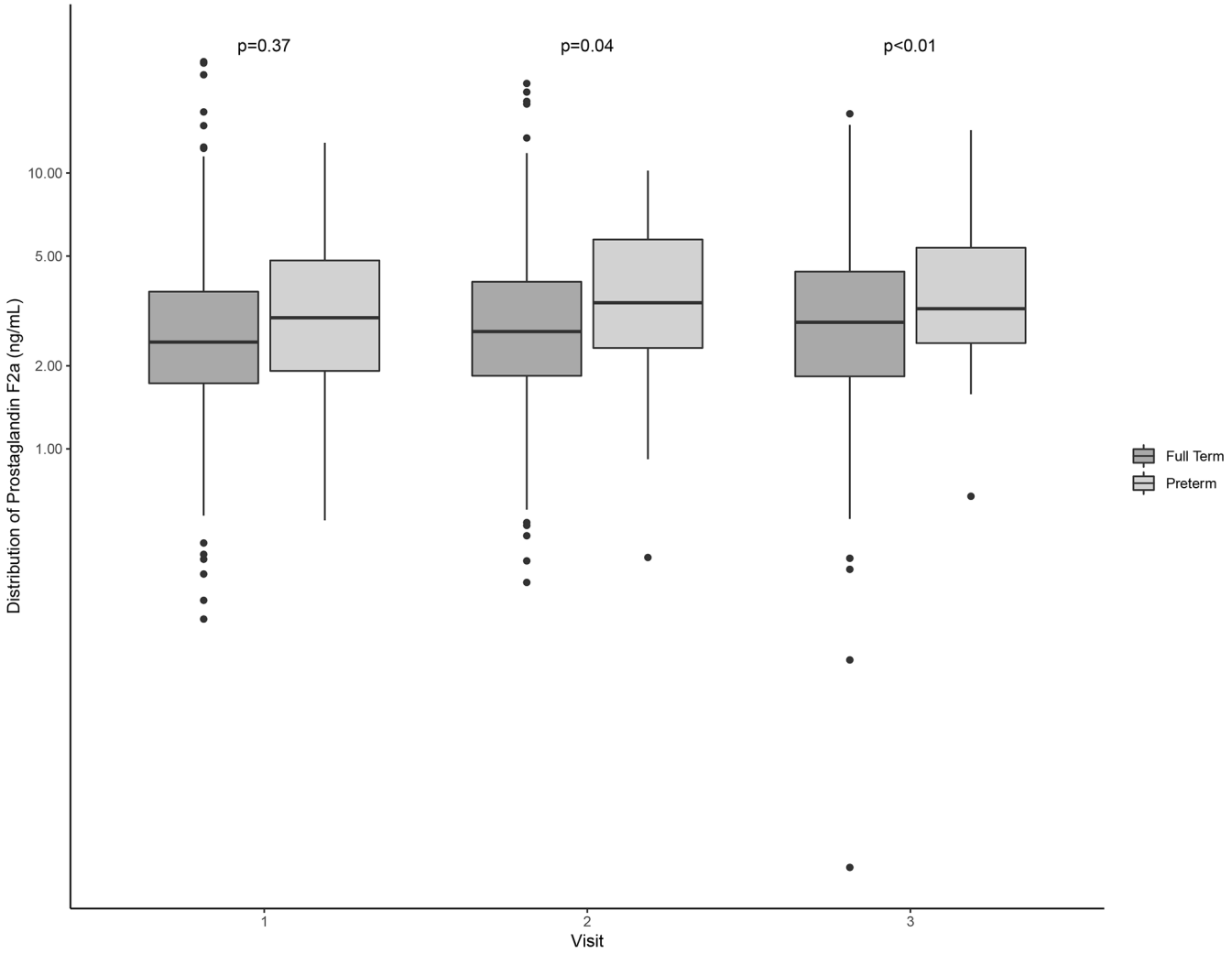

Figure 3.

Distribution of specific gravity-corrected urinary levels of $PGF_{2\alpha}$ (ng/mL) by study visits stratified by preterm (gestational age <37 weeks) and full term birth (gestational age ≥37 weeks).

Note: The mean gestational age at study visit was 18, 24, and 28 weeks gestation, respectively. P-values were calculated using t-tests and $p<0.05$ indicates statistical significance.

Table 1.

Distributions of characteristics in the PROTECT analytic sample (N=469).

Characteristic	N (%)
Preterm Birth	
Preterm	50 (11.2)
Full Term	396 (88.8)
Missing	0 (0.00)
Maternal Age, years	
18-24	188 (40.1)
25-29	142 (30.3)
30-34	85 (18.1)
≥35	53 (11.3)
Missing	1 (0.21)
Maternal Education	
<High school	35 (7.46)
High school or equivalent	70 (14.9)
Some college or technical school	167 (35.6)
≥College degree	193 (41.5)
Missing	4 (0.85)
Employment Status	
Unemployed	176 (37.5)
Employed	288 (62.4)
Missing	5 (1.07)
Marital Status	
Single	89 (19.0)
Married	250 (53.3)
Living together	128 (27.3)
Missing	2 (0.43)
Alcohol Use	
Never	204 (43.5)
Before pregnancy	234 (49.9)
Currently	25 (5.33)
Missing	6 (1.28)
Smoking	
Never	385 (82.1)
Before pregnancy	63 (13.4)
Currently	19 (4.05)
Missing	2 (0.43)
Insurance Status	

Characteristic	N (%)
Private	281 (59.9)
Public	165 (35.2)
Uninsured	6 (1.28)
Missing	17 (3.62)

Note: percentages may not sum to 100% due to rounding; missing indicates the number and percent of participants for which information regarding the specific characteristic was not obtained.

Table 2.

Distribution of subject-specific urinary levels of oxidative stress biomarkers corrected with specific gravity (ng/mL).

			Percentile				
	N	**Geometric Mean (Geometric SD)**	**5**	**25**	**50**	**75**	**95**
Measured							
8-iso-$PGF_{2\alpha}$	1,146	1.84 (1.66)	0.83	1.34	1.86	2.53	4.14
8-iso-$PGF_{2\alpha}$ metabolite	1,146	0.88 (1.72)	0.38	0.63	0.87	1.26	2.10
Prostaglandin-$F_{2\alpha}$	1,146	2.71 (1.94)	0.93	1.84	2.72	4.07	7.85
Derived							
8-iso-$PGF_{2\alpha}$ chemical	1,146	1.43 (1.86)	0.53	0.98	1.45	2.18	3.57
8-iso-$PGF_{2\alpha}$ enzymatic	1,146	0.10 (10.9)	0.00	0.02	0.29	0.57	1.16

Abbreviations: SD, standard deviation.

Note: there were 270 samples from visit 1, 349 from visit 2, and 217 from visit 3. Measured biomarkers were analyzed in urine. Derived biomarkers were quantified using the ratio of 8-iso-$PGF_{2\alpha}$ to $PGF_{2\alpha}$ to determine the proportion of 8-iso-$PGF_{2\alpha}$ that was derived from chemical and enzymatic lipid peroxidation pathways.

Table 3.

Crude and adjusted odds ratios (95% confidence interval) for preterm birth with an interquartile range increase in specific gravity-corrected urinary oxidative stress biomarker (ng/mL) averaged across pregnancy (visits 1-3) and by study visit.

	Crude			Adjusted[1]		
Biomarker	**N (PTB, FTB)**	**OR (95% CI)**	**p-value**	**N (PTB, FTB)**	**OR (95% CI)**	**p-value**
Measured						
8-iso-$PGF_{2\alpha}$						
Average	(50, 396)	1.60 (1.06, 2.43)	0.03	(50, 393)	1.64 (1.07, 2.54)	0.02
Visit 1	(31, 220)	1.16 (0.70, 1.91)	0.57	(30, 214)	1.11 (0.65, 1.88)	0.71
Visit 2	(34, 305)	1.49 (0.93, 2.41)	0.10	(34, 298)	1.55 (0.95, 2.55)	0.08
Visit 3	(24, 187)	1.92 (0.98, 3.93)	0.06	(24, 179)	1.77 (0.87, 3.80)	0.13
8-iso-$PGF_{2\alpha}$ metabolite						
Average	(50, 396)	1.76 (1.15, 2.71)	<0.01	(50, 393)	1.79 (1.14, 2.84)	0.01
Visit 1	(31, 220)	1.10 (0.64, 1.89)	0.72	(30, 214)	1.19 (0.66, 2.14)	0.57
Visit 2	(34, 305)	1.33 (0.83, 2.14)	0.23	(34, 298)	1.43 (0.88, 2.34)	0.15
Visit 3	(24, 187)	3.73 (1.96, 7.66)	<0.01	(24, 179)	3.56 (1.78, 7.69)	<0.01
Prostaglandin-$F_{2\alpha}$						
Average	(50, 396)	1.88 (1.28, 2.80)	<0.01	(50, 393)	1.98 (1.32, 3.02)	<0.01
Visit 1	(31, 217)	1.24 (0.79, 1.92)	0.35	(30, 211)	1.24 (0.78, 1.96)	0.36
Visit 2	(34, 303)	1.57 (1.01, 2.48)	0.05	(34, 296)	1.60 (1.01, 2.58)	0.05
Visit 3	(24, 187)	2.27 (1.18, 4.64)	0.02	(24, 179)	2.01 (1.03, 4.17)	0.05
Derived						
8-iso-$PGF_{2\alpha}$ chemical						
Average	(50, 396)	1.17 (0.91, 1.52)	0.24	(50, 393)	1.17 (0.90, 1.54)	0.25
Visit 1	(31, 217)	1.07 (0.81, 1.45)	0.65	(30, 211)	1.03 (0.76, 1.43)	0.85
Visit 2	(34, 303)	1.15 (0.86, 1.56)	0.37	(34, 296)	1.18 (0.87, 1.62)	0.29
Visit 3	(24, 187)	1.22 (0.84, 1.82)	0.31	(24, 179)	1.19 (0.81, 1.82)	0.39
8-iso-$PGF_{2\alpha}$ enzymatic						
Average	(50, 396)	1.69 (1.07, 2.84)	0.04	(50, 393)	1.73 (1.09, 2.93)	0.03
Visit 1	(31, 217)	1.63 (0.68, 4.29)	0.30	(30, 211)	1.62 (0.67, 4.33)	0.30
Visit 2	(34, 303)	1.45 (1.00, 2.28)	0.08	(34, 296)	1.45 (1.00, 2.29)	0.07
Visit 3	(24, 187)	1.43 (0.89 2.60)	0.18	(24, 179)	1.42 (0.88, 2.54)	0.19

[1] Average models adjusted for maternal age, education, and marital status. Visit 1, 2, and 3 models adjusted for maternal age, education, marital status, and gestational age at visit.

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

Note: average indicates biomarker concentrations that were obtained by taking the geometric mean of the available specific gravity-corrected oxidative stress biomarker concentrations from all participants at visits 1-3.