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Amniotic Fluid Eicosanoids in Preterm and Term Births: Effects of Risk Factors for Spontaneous Preterm Labor

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Abstract

OBJECTIVE—To evaluate amniotic fluid (AF) arachidonic acid metabolites using enzymatic and nonenzymatic (lipid peroxidation) pathways in spontaneous preterm birth and term births, and to estimate whether prostanoid concentrations correlate with risk factors (race, cigarette smoking, and microbial invasion of amniotic cavity) associated with preterm birth.

METHODS—In a case-control study, AF was collected at the time of labor or during cesarean delivery. AF samples were subjected to gas chromatography, negative ion chemical ionization, and mass spectrometry for prostaglandin (PG)E₂, PGF_{2α}, and PGD₂, 6-keto-PGF_{1α} (6-KPGF_{1α}, thromboxane (TXB₂), and F₂-isoprostane (F₂-IsoP). Primary analysis examined differences between prostanoid concentrations in preterm birth (n=133) compared with term births (n=189). Secondary stratified analyses (by race, cigarette smoking and microbial invasion of amniotic cavity) compared eicosanoid concentrations in three epidemiological risk factors.

RESULTS—AF F₂-IsoP, PGE₂, and PGD₂ were significantly higher at term than in PTB, whereas PGF_{2α} was higher in PTB. 6-KPGF_{1α} and TXB₂ concentrations were not different. Data stratified by race (African American or Caucasian) showed no significant disparity among prostanoid concentrations. Regardless of gestational age status, F₂-IsoP was threefold higher in smokers, and other eicosanoids were also higher in smokers compared to non-smokers. Preterm birth with microbial invasion of amniotic cavity had significantly higher F₂-IsoP compared to preterm birth without microbial invasion of amniotic cavity.

CONCLUSIONS—Most AF eicosanoid concentrations (F₂-isoP PGE₂ and PGD₂), are higher at term than in preterm birth. The only AF eicosanoid that is not higher at term is PGF_{2α}.

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INTRODUCTION

Despite remarkable advances in general medical care, the preterm birth rate has increased in the United States, rising by as much as 30% during the last twenty-five years. (1-3) Racial disparity in PTB, a long recognized risk factor, also appears to be widening, further complicating our understanding of its pathophysiologic manifestations. (1,2,4) The current management of spontaneous preterm labor resulting in preterm birth (PTB) is based on a universal strategy to inhibit uterine contractions in a large subset of cases (especially < 34 weeks) without proper identification of risk factors, understanding of specific initiators and effectors or knowledge of responsible pathophysiologic pathways in subjects who typically have multiple exposures.(4)

PTB dogma invokes a linear mechanistic pathway, with several putative etiologic factors (e.g., infection, cigarette smoking, pPROM, coagulation disorders, uterine distension, and behavioral or psychosocial stress) converging on an inflammatory reaction mediated by cytokines and matrix metalloproteinases, ultimately precipitating prostaglandin [PG] generation, from cell membrane derived arachidonic acid (AA) metabolism. $\text{PGF}_2\alpha$ and PGE_2 are the predominant and most studied PGs associated with PTB since they can cause cervical ripening and dilatation, membrane weakening and rupture, and stimulate myometrial contractility resulting in labor. (5-13) Due to their uterotonic activities, PGs have been postulated as the final effector molecules of labor, although the initiating signals of these events may differ in preterm and term deliveries. Inflammation has been documented as a manifestation of both preterm and term labor, but targeting PG synthesis as a first line intervention has not been successful in reducing the rate of PTB. The limited success of PG inhibitors to prevent PTB may reflect a failure to precisely identify the underlying causal or effector pathway(s) involved. It is possible that an alternate pathway(s) of labor exists, possibly mediated by less well characterized eicosanoids.

Oxidative stress has been suggested as a mediator of labor initiation in response to certain risk exposures. (14, 15) Oxidative stress in pregnancy arises when the production of reactive oxygen species (ROS) exceeds the capacity of antioxidant stores. Cigarette smoking, nutrient deficiencies, high energy demands of the fetoplacental unit, intrinsic microvascular disease, anaerobic infections and placental apoptosis, can result in an imbalanced redox state. While inflammation and oxidative stress may share some risk factors and mechanistic pathways, we postulate that different mediators are involved. Non-enzymatic oxidation of AA can result in the production of isoprostanes (IsoP), whereas cytokine activation secondary to inflammation yields enzymatically produced PGs ($\text{PGF}_2\alpha$, PGE_2 , and PGD_2), thromboxane B2 (TxB2) and the prostacyclin metabolite 6-keto- $\text{PGF}_1\alpha$ (6-KPGF1 α). (16-18) The best studied IsoP form, 8-epi- or F2-IsoP, contains F-type prostane rings isomeric to $\text{PGF}_2\alpha$ (16-18) Biological functions of IsoPs are tissue, cell, and concentration dependent (16-19) and include smooth muscle constriction, macrophage activation, vascular cell proliferation and induction of endothelin-1 (ET-1) release.(16-19) The latter is a potent uterotonic known to be produced by fetal membranes.(20-22)

The roles of eicosanoids besides $\text{PGF}_2\alpha$ and PGE_2 have neither been well characterized nor proposed as alternate mediators of PTB or term birth. In fact, even the role of PGE_2 has been questioned in a recent report showing that amnion production of PGE_2 was not associated with PTB.(23) To obtain eicosanoid signatures in preterm and term deliveries, we measured F2-IsoP, $\text{PGF}_2\alpha$, PGE_2 , PGD_2 , 6-KPGF1 α and TxB2 in amniotic fluid (AF) from PTB (cases) and normal term births (controls) using a specific and sensitive gas chromatography/negative ion chemical ionization mass spectrometry (GC/NICI/MS) method. Secondary analyses were performed on stratified data to document the effect of three risk factors of PTB: race, cigarette smoking and intraamniotic infection or microbial invasion of the

amniotic cavity (MIAC), which are reported to modify pregnancy outcome (PTB vs. normal term birth). Our findings indicate that F2-IsoP represents a biomarker for an alternate pathway mediating a subgroup of PTB cases.

METHODS

This study was conducted at Centennial Women's Hospital, Nashville, TN and Emory University, Atlanta, GA and was approved by the TriStar Nashville institutional review board (Centennial Medical Center), Western Institutional Review Board (Seattle, WA) and the Emory University IRB (Atlanta, GA). All subjects were recruited at Centennial Women's Hospital between September 2003 and December 2008, assays were performed at Vanderbilt University Eicosanoid Core Laboratory, study design, selection of parameters and data analysis were conducted at Emory University.

In this cross – sectional study of a retrospective cohort, pregnant women between the ages of 18 and 40 presenting to Centennial Women's Hospital, Nashville, TN, for delivery were eligible to consent for this study. Subjects were enrolled after obtaining written consent. Amniotic fluids were collected and eicosanoids were measured on samples from each group based solely on the order of recruitment into the study. (23-27) All subjects had spontaneous labor (defined as the presence of regular uterine contractions at a minimum frequency of 2 contractions/10 minutes) that led to delivery. Race was identified by self-report and determined by the race of the mother and father of the fetus, their parents and grandparents. If subjects reported any family members different from the rest, they were excluded (e.g. African-American when the rest were Caucasian or vice versa).. (24-28) Gestational age was determined by last menstrual period dating, corroborated by ultrasound. Patients with spontaneous onset of labor who delivered preterm (between 24^{0/7} weeks and 36^{6/7} weeks) were considered as cases. Control subjects were chosen based on a normal pregnancy ending in term labor and delivery ($\geq 37^{0/7}$ weeks) who had intact membranes and no pregnancy-related complications or prior history of pregnancy complications including preterm labor and pPROM. These criteria were used to exclude overlap between cases and controls. Subjects with multiple gestations, preeclampsia, placental previa, preterm prelabor rupture of the membranes, fetal anomalies, gestational diabetes mellitus or other medical/surgical complications of pregnancy were excluded. Subjects who were treated for preterm labor or for suspected intraamniotic infection and delivered at term were excluded from the control group; but those who were treated and delivered preterm were included as cases.

Demographic data were collected from interviews and clinical data were abstracted from subjects' medical records. Age, socioeconomic data (education, yearly income, insurance status and marital status), behavioral status (smoking during pregnancy), body mass index, and a complete medical and obstetric history were collected.

For vaginal deliveries, amniotic fluid samples were collected during labor (either preterm or term) immediately before artificial rupture of the membranes by transvaginal amniocentesis of intact membranes using a 22 gauge needle through the dilated cervical os. Samples were also collected by placement of an intrauterine pressure catheter in a few cases where indicated for clinical reasons. . This procedure avoided contamination of amniotic fluid from vaginal and cervical fluids as verified by analysis for MIAC. In cases undergoing cesarean delivery, samples were collected by transabdominal amniocentesis. Amniotic fluid was centrifuged immediately for 10 minutes at 2000 g to remove cellular and particulate matter and supernatant aliquots were processed rapidly and stored in the dark at -80°C in filled tubes to minimize artifactual oxidation until analysis. As no pre-existing data were available to estimate power to detect differences between races we studied the first 150 African American and 177 Caucasian samples collected. In post-hoc analysis, we determined that

133 cases and 169 controls provided > 80% power to detect the differences in analyte concentrations between the groups at a 0.05 significance level for each analyte in their log-transformed form.

Microscopic examination of the placenta and umbilical cord was performed on all cases. Histologic evidence of chorioamnionitis was defined as a dense polymorphonuclear leukocyte/neutrophil infiltration of the amniochorionic membrane (excluding decidua), and funisitis was defined as inflammation in one or more of the umbilical cord vessels with or without inflammation in the Wharton's jelly. Clinical chorioamnionitis (modified from Duff et al) (30) was defined as any three of the following: increased white blood cell count >15,000/dL, increased C-reactive protein levels (≥ 0.8 Units/mL), fever ($\geq 102.0^{\circ}\text{F}$), abdominal pain or uterine tenderness and foul smelling vaginal discharge as verified from case records. Bacterial vaginosis was diagnosed using Nugent's criteria on Gram stained vaginal swabs, although its specificity has been challenged (REF). Microbial invasion of the amniotic cavity (MIAC) was defined by the presence of bacteria in the amniotic fluid detected by amplification of microbial 16s ribosomal DNA by polymerase chain reaction (PCR) (TaqMan Assay, CA). (31, 32) MIAC was determined by either PCR or by microbial cultures in all samples from cases and in a subgroup of (n=50) randomly selected control samples collected by transvaginal amniocentesis (from both races) to rule out contamination of AF during specimen collection.

All samples included in this study were assayed simultaneously to avoid any variability introduced during assay procedures. AF samples (0.20 mL) were diluted to a volume of 10 mL with 0.01N HCl and 1.0 ng of each of the following internal standards were added to the solution; [$^2\text{H}_4$]-15-F₂-IsoP ([$^2\text{H}_4$]-8-iso-PGF_{2 α}), [$^2\text{H}_4$]-PGD₂, [$^2\text{H}_4$]-PGE₂, [$^2\text{H}_3$]-11-dehydro-thromboxane B₂ (11-dehydro-TxB₂), and [$^2\text{H}_4$]-6KPGF1 α (all purchased from Cayman Chemicals, Ann Arbor, MI). The details of this method can be seen in our prior publications (33).

GC/NICI/MS was carried out on an Agilent 5973 Inert Mass Selective Detector interfaced with a computerized Agilent 6890n Network GC system. The GC phase was performed using a 15 m, 0.25 mm film thickness, DB-1701 fused silica capillary column (J and W Scientific, Folsom CA). The column temperature was programmed from 190° to 300°C at 20°C increments per minute. Levels of endogenous eicosanoids in the biological samples were calculated from the ratio of intensities of the [$^2\text{H}_0$]- and [$^2\text{H}_4$]-ions. GC/NICI/MS allowed us to measure six different analytes in the same AF sample in a single run and provided a more sensitive and accurate measurement of these analytes than ELISA.

The summary statistics for amniotic fluid F₂-IsoP and prostaglandin concentrations between cases and controls stratified by race are displayed in Table 2. [F₂-IsoP and PGF₂ were measurable in 99% of cases (133/134) and 98% (189/193) controls. PGE_{2 α} was measurable in 89% (119/134) of cases and 98% (189/193) controls. 6-KPGF1 α was measured in 69% (92/134) cases and 89% (171/194) controls. TxB₂ was detected in 85% (114/134) of cases and 93% of controls. PGD₂ was obtained only in 63% (84/134) cases and 86% controls (166/194).] The limit of detection for all analytes was 2 pg/ml and (non measurable) indicates that no peaks were observed in some samples for some of the analytes.

Data are presented as mean $\pm$ SD. For continuous and categorical variables, respectively, Student *t* tests and chi-square tests (Fisher Exact test for bacterial vaginosis) were performed to determine the statistical significance of any differences in the distribution of baseline characteristics between preterm birth cases and controls, overall and stratified by race. Normality was tested using Kolmogorov-Smirnov test prior to performing statistical analysis. Because the analyte concentrations were not normally distributed, log-

transformation was performed to justify the use of Student's *t*-test to compare concentrations among cases and controls and also between races. Two-tailed $P=0.05$ was considered the threshold for statistical significance. Primary analysis of differences in prostanoid concentrations between cases and controls documented their distributions during preterm and term births. Secondary analyses were utilized to compare differences in analytes between races, between smokers vs. non-smokers and in cases complicated by MIAC.

RESULTS

Of the 327 participating women (150 African Americans and 177 Caucasians), 134 subjects had PTB (cases) and 193 normal term deliveries (controls). Table 1 shows selected maternal characteristics and pregnancy outcomes in cases and controls, stratified by race.

Demographic and clinical data are based on available information, as all endpoints were not available from every participant. No significant differences between cases and controls were observed for infant sex, marital status, smoking status, annual income, body mass index, and gravidity for both races. Among Caucasians, the mean age of women delivering preterm was two years younger than that of women with normal term births (cases = 26.8 ± 6.1 years; controls = 28.7 ± 5.8 years, $p=0.04$), and the proportion of women with $\leq$ high school education was higher in cases (55.8%) than controls (32.6%, $p = 0.002$). Among cases, no significant differences between the two racial groups were seen in maternal age ($p = 0.08$), gestational age ($p = 0.55$), birth weight ($p = 0.56$), Apgar at 1 minute ($p = 0.38$) or latency ($p = 0.10$). In controls, maternal age (25.2 ± 5.5 vs. 28.7 ± 5.8 years), birthweight (3247 ± 399 vs. 3386 ± 470 g) were significantly lower in blacks ($p<0.007$). In both races, the proportion of women with bacterial vaginosis (based on Nugent's scores) was significantly greater among cases than in controls ($p = 0.001$). (Table 1) The prevalence of MIAC with histologic chorioamnionitis (20% in African Americans and 26% in Caucasians) and funisitis (6% in African Americans and 1% in Caucasians) in PTB were not significantly different between the two races.

Data presented here are log transformed mean $\pm$ ST. In combined data analysis, PTB had significantly lower mean concentrations of F2-IsoP (0.34 ± 0.26 ng/ml) than controls (1.30 ± 0.69 ng/ml, $p = 0.003$). Racial disparity was not evident in our stratified analysis. Cases in African Americans (0.32 ± 0.20 ng/ml) and in Caucasians (0.36 ± 0.29 ng/ml) had significantly lower F2-IsoP than their respective controls (African American controls $0.46 \pm .38$ ng/ml; $p 0.01$, Caucasian controls - 0.45 ± 0.31 ng/ml; $p = 0.008$) (Table 2). $\text{PGF}_{2\alpha}$ concentrations were higher in cases than controls in combined data analysis (11.34 ± 13.74 ng/ml vs. 6.63 ± 8.04 ng/ml, $p = 0.004$), among African Americans (11.39 ± 14.13 ng/ml vs. 6.93 ± 8.19 ng/ml, $p=0.034$) and in Caucasians (11.31 ± 13.55 ng/ml vs. 6.35 ± 7.92 ng/ml in controls, $p = 0.004$). Similar to F2-IsoP, PGE_2 concentrations were significantly lower in cases overall (4.36 ± 6.38 vs. 7.20 ± 8.94 ng/ml, $p < 0.001$) and racial disparity was also not evident with PGE_2 although the statistical significance in Africans were marginal. In Caucasians PGE_2 concentrations were 3.04 ± 4.02 in cases and 7.18 ± 8.07 ng/ml in controls ($p < 0.0001$) and in African Americans (6.00 ± 8.18 vs. 7.22 ± 9.83 ng/ml; $p = 0.08$). Similarly overall PGD_2 concentrations were significantly lower in cases than in controls (1.53 ± 1.72 ng/ml) vs. 2.89 ± 4.03 ng/ml; $p = 0.0001$) regardless of race (African American cases 1.72 ± 1.72 ng/ml; controls 3.22 ± 4.74 ng/ml; $p = 0.01$ and Caucasian cases 1.31 ± 1.71 ng/ml; controls 2.63 ± 3.37 ng/ml, $p = 0.0005$). Concentrations of 6-KPGF1 α and TxB_2 between cases and controls were not different in combined or stratified analysis.

The secondary objective of this study was to address three major risk factors associated with PTB and their effects on eicosanoid concentrations. Race, cigarette smoking and MIAC are factors previously documented to be associated with early delivery and adverse pregnancy outcomes. Data were stratified based on these factors in the following analyses.

Among cases, comparisons of the concentrations of eicosanoid analytes between African Americans and Caucasians showed no significant differences (Table 3) with the exception of PGE₂, which doubled in African American (6.00 ± 8.19 ng/ml) compared to Caucasian (3.04 ± 4.02 ng/ml) with a marginal level of significance ($p = 0.07$) in cases. No differences among any of the analytes were noted between races in controls. In general, amniotic fluid prostanoid concentrations in PTB or term births did not reveal evidence of racial disparity.

In order to document the effects of cigarette smoking, a known inducer of oxidative stress, we analyzed AF eicosanoids after stratification for this behavior (Table 4). Although all eicosanoids were increased, F2-IsoP was increased by ~ 3 fold in smokers compared to non smokers regardless of pregnancy status (preterm or term). F2-IsoP was significantly higher in smokers with PTB (smokers 0.60 ± 0.28 ng/ml vs. non smokers 0.26 ± 0.19 ng/ml; $p < 0.0001$) and also at term (smokers 0.92 ± 0.39 ng/ml vs. nonsmokers 0.37 ± 0.26 ng/ml; $p = < 0.0001$). Data further stratified by race showed no racial disparity in F2-IsoP concentrations in either cases or controls (Table 5). In a combined or stratified analysis, PGF₂ α concentration was not significantly different among normal term births regardless of smoking status ($p = 0.213$); however, > 2 fold more PGF₂ α was seen in smokers compared to non smokers ($p < 0.0001$) in PTB group. PGE₂, PGD₂ and 6-KPGF₁ α were also higher among smokers compared to non smokers regardless of pregnancy status (case or control). Data stratified by race showed increased PGE₂ in African American smokers compared to non smokers in both cases ($p < 0.0001$) and controls ($P = 0.04$). This was also evident in Caucasian smokers in normal term birth group compared to non smokers ($p = 0.002$) but not in PTB group ($p = 0.48$). Similar trends were evident with PGD₂, and 6-KPGF₁ α . TxB₂ was higher in smokers from normal term births compared to non smokers in this group ($p = 0.001$) but not in PTB group. Only smokers in Caucasian normal term birth group had higher TxB₂ compared to non smokers but it was not different in African Americans. Finally we examined the role of MIAC in AF prostanoid concentrations. This aspect of the study was limited to PTB only, as infection or antibiotic treatment during pregnancy were exclusion criteria for our controls. 29 cases with MIAC were identified (Table 6). Overall, F2-IsoP was marginally higher among cases with infection (0.45 ± 0.35 ng/ml) compared to controls (0.31 ± 0.22 ng/ml; $p = 0.02$). Data stratified by race showed that Caucasian cases with MIAC had higher F2-IsoP compared to cases without MIAC ($p = 0.008$) (Table 7). None of the other eicosanoids showed any differences between cases with and without MIAC. Although higher concentrations of PGF₂ α were seen in both African American and Caucasian cases with MIAC compared to those with no MIAC, none of these data reached statistical significances in either races, likely due to sample size and variability ($p = 0.88$ and 0.65 respectively).

DISCUSSION

Using a very sensitive and specific mass spectrometry assay, we profiled six different eicosanoids in AF from preterm and term births. Our primary objective was to document the differential accumulation of variably bioactive eicosanoids and related AA metabolites under well characterized clinical conditions. Based on our primary analysis we discovered: 1) measurable quantities of six different eicosanoids in the AF samples of both preterm and term births; 2) PGF₂ α is the only AF eicosanoid that is not higher at term; 3) In support of recent reports from Romero's group, we also observed that PGE₂ and PGD₂ concentrations are ~2 fold higher at term than preterm, implying that these eicosanoids are more likely to play a role in physiological parturition than in PTB; and 4) that F2-IsoP can be detected in preterm and term AF in pregnancies with intact membranes. This is the first report of the latter observation; 5) Higher F2-IsoP concentrations in AF at term suggest that oxidative stress is a contributor to normal parturition; and 6) 6-KPGF₁ α and TxB₂ concentrations do not appear to differ between preterm and term births.

Secondary analyses were performed to examine AF eicosanoid signatures based on three classical risk factors for PTB: race, cigarette smoking and MIAC. Data stratified by race showed no racial disparity in the pattern of eicosanoids, with the exception that higher PGE₂ concentrations were noted in African Americans with PTB. Cigarette smoking, a known inducer of oxidative stress, was found to be associated with high concentrations of F2-IsoP. Regardless of the pregnancy status and race, smokers had ~3-fold higher F2-IsoP levels, confirming its role as a biomarker of oxidative stress. Cigarette smoking shows a generalized increase in other eicosanoids. High F2-IsoP in normal term deliveries suggests that oxidative stress is a physiological precursor or consequence of labor. Infection was also associated with higher F2-IsoP and PGF₂ α in cases. The data on PGF₂ α confirm those already reported by other groups; however, the F2-IsoP findings are novel with respect to intraamniotic infection and suggest oxidative stress as a mechanism in MIAC-induced PTB. Contrary to prior reports, we did not see any difference in PGE₂ concentrations and infection. Amniotic fluid 6KPGF1 α TxB2 and PGD₂ also showed no relationship to infection status.

One of the major findings of this study is the documentation of substantially increased oxidative stress during normal labor and delivery at term. Oxidative stress previously has been hypothesized as a mediator of PTB, but biomarkers of oxidative stress have not been reported in preterm nor term AF. Placental and nutrient antioxidants like superoxide dismutase, catalase, and glutathione peroxidase maintain AF redox status during pregnancy. Although there is an overall increase in antioxidant over pro-oxidant activity as gestation advances, antioxidant production is not sufficient to balance oxidative stress once gestation reaches term. (34, 35) Therefore we suggest that oxidative stress exceeds a threshold triggering the onset of labor when gestation and fetal growth are complete. Oxidative stress at term and during labor could result from multiple mechanisms: increased maternal and fetal physiological stress, increased mitochondrial activity due to high energy demand associated with labor, and increased apoptosis among placental, fetal membrane and decidual cells. Apoptosis and oxidative stress should be considered as natural physiological responses at term due to aging of the fetoplacental unit. In addition, maternal-fetal signals favoring lipid peroxidation may promote production of inflammatory or uterotonic eicosanoids (e.g., PGF₂ α) and induce labor. Other AA metabolites, such as F2-IsoP, which are not known to have intrinsic uterotonic properties, may have indirect effects on labor, e.g. by increasing endothelin production, a known inducer of myometrial contractility. (36-39) To confirm that oxidative stress is a labor associated change, we compared AF F2-IsoP from subjects not in labor undergoing cesarean sections to laboring women at term having normal vaginal deliveries. The latter had significantly higher AF F2-IsoP concentrations (0.52 ± 0.37 ng/ml) than the former subjects (0.36 ± 0.30 ; $p = 0.004$). Higher F2-IsoP levels also were noted in cigarette smokers (regardless of race or pregnancy status), confirming the intrauterine response to this well-documented provocation of oxidative stress. These data from cigarette smokers are supported by our *in vitro* data where cigarette smoke extract-stimulated normal term fetal membrane explants secrete more F2-IsoP than vehicle-stimulated membranes (33).

As it is well known that many cases of PTB have an inflammatory basis, we were not surprised by the elevated AF PGF₂ α levels noted in this subgroup. However we were impressed by the lack of oxidative stress-associated biomarker (F2-IsoP) in PTB with intact membranes relative to births at term. However, increased F2-IsoP in PTB was observed in pregnancies complicated by cigarette smoking or infection. These results prompt us to postulate that oxidative stress, as manifested by F2-IsoP, is a risk specific response to smoking and infection but not necessarily a general effector of labor process. While considerable overlap and interaction exist between the inflammatory and oxidative stress pathways, our results suggest that these pathways may uniquely be operative in different

pregnancy complications and their activation is likely dependent on the type of risk exposure.

Our study also rules out PGE₂ and PGD₂ as eicosanoids significantly associated with preterm labor. (23, 40-46) Further risk modifiers including race, cigarette smoking, and infection also were unrelated to PGE₂ and PGD₂ concentrations in PTB. Differential effects on labor by PGF₂ α and PGE₂ have been reported previously, based on different PG receptors and modes of signal transduction. [47,48] Our findings confirm those of Romero et al that PGF₂ α is the dominant uterotonic PG. [41] 6-KPGF₁ α and TxB₂, markers of vasodilation and vasoconstriction, respectively, have been invoked in pregnancy complications like preeclampsia, but our data indicate that they are unlikely to be involved in PTB. (49-51)

The use of mass spectrometric methods allowed us to avoid the cross-reactivity and lack of specificity associated with classical immunological methods to detect these structurally similar compounds used in prior publications (52). In summary, coordinated (but still unclear) interactions among eicosanoid mediators of inflammation and oxidative stress are physiological signals of maturation and labor and appear to be pathophysiologically induced by some risk exposures in PTB. We report that term birth is associated with oxidative stress as evidenced by increased AF F₂-IsoP and that certain risk categories of PTB also are associated with oxidative stress. These chemically related effectors are unlikely independent in their action. This hypothesis partly explains the failure of a single intervention (e.g., COX inhibition) to be effective in all subjects with preterm labor. Our secondary analyses document the influence of specific risk factors of spontaneous PTB on AF prostanoid levels and support oxidative stress as an underlying mechanistic factor in cases involving cigarette smoking and MIAC. Depending on individual's own risk exposures that can cause inflammation or oxidative stress (infection, behavioral, nutritional deficiencies, BMI, psychosocial and socio-economic stressors, genetic, race etc.), pathophysiologic manifestations can vary from subject to subject. In summary, biomarkers and mechanistic effectors of preterm and term labor are not universal; hence clinical interventions in preterm birth cannot be generalized. Depending on an individual's unique risk factors, pathophysiologic manifestations can vary from subject to subject. This report supports the hypothesis that alternate pathways and biomarkers need to be considered in the etiology and individualized interventions tailored for effective treatment of preterm labor.

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Maternal characteristics and pregnancy outcomes between preterm birth and term births stratified by race

TABLE 1

Characteristics	African Americans (n = 150)			Caucasians (n = 177)			Total (n = 327)	
	Cases (n = 56)	Controls (n = 94)	P- value	Cases (n = 78)	Controls (n = 99)	P- value	Cases (n = 134)	Controls (n = 193)
Gestation (weeks)	34.1 ± 2.6	38.0 ± 6.1	<0.001	32.9 ± 3.4	38.4 ± 4.1	<0.001	33.4 ± 3.1	38.2 ± 5.1
Range	24.1-36.1	37.0-41.7		23.7-36.6	37.0-41.0		23.7-36.6	35.7-41.7
Birthweight (g)	2246.7 ± 622.6	3247 ± 399.0	<0.001	2016.9 ± 778.2	3386.8 ± 470.1	<0.001	2120.6 ± 718.4	3323.6 ± 443.4
Maternal Age	26.5 ± 5.0	25.5 ± 5.5	0.28	26.8 ± 6.1	28.7 ± 5.8	0.04	26.7 ± 5.7	27.1 ± 5.9
Married (%)	20/52 (38.5)	25/90 (27.8)	0.19	46/75 (61.3)	73/98 (74.5)	0.064	66/127 (52.0)	98/188 (52.1)
Smoker (%)	9/56 (16.1)	17/94 (18.1)	0.75	20/78 (25.6)	18/99 (18.2)	0.23	29/134 (21.6)	35/193 (18.1)
Annual Income < \$50,000 (%)	48/54 (88.9)	78/90 (86.7)	0.7	49/75 (65.3)	64/97 (66.0)	0.93	97/129 (75.2)	142/187 (75.9)
Education ≤ 12 years (%)	32/54 (59.2)	59/90 (65.6)	0.45	43/77 (55.8)	32/98 (32.6)	0.002	75/131 (57.3)	91/188 (48.4)
BMI	28.6 ± 8.3	28.8 ± 7.9	0.9	26.4 ± 7.1	26.4 ± 6.9	0.97	27.4 ± 7.6	27.6 ± 7.5
Apgar-1	7.3 ± 1.4	8.1 ± 1.1	<0.001	7.2 ± 1.7	8.5 ± 0.8	<0.001	7.3 ± 1.6	8.3 ± 1.0
Apgar-5	8.6 ± 0.7	9.0 ± 0.3	<0.001	8.4 ± 1.1	9.0 ± 0.2	<0.001	8.5 ± 1.0	9.0 ± 0.2
Gravidity	3.2 ± 1.8	2.7 ± 1.4	0.09	2.1 ± 1.3	2.5 ± 1.4	0.08	2.5 ± 1.6	2.6 ± 1.4
Latency (days)**	5.3 ± 13.3			4.1 ± 5.7			4.6 ± 9.7	
Infant Female (%)	31/54 (57.4)	35/81 (43.2)	0.11	31/69 (44.9)	55/91 (60.4)	0.05	62/123 (50.4)	90/172 (52.3)

Characteristics	African Americans (n = 150)		Caucasians (n = 177)		Total (n = 327)	
	Cases (n = 56)	Controls (n = 94)	P- value	Cases (n = 78)	Controls (n = 99)	P- value
Bacterial Vaginosis (%)	9/56 (16.1)	5/89 (5.6)	0.04	8/77 (10.4)	1/98 (1.0)	0.01
				17/133 (12.8)	6/187 (3.2)	0.001

Data shown here are based on available information. Not all cases and controls are included. Total samples analyzed 327.

** - All controls had spontaneous labor followed by delivery and the latency ranges between 1 hour to a maximum of 24 hours. Therefore no comparisons were made.

TABLE 2

Comparison of amniotic fluid concentrations of F2-IsoP, PGF_{2α}, PGE₂, 6-KPGF1α, TxB2 and PGD2 between Caucasians and African Americans among preterm and normal term deliveries

Parameters	African Americans				Caucasians				Total	
	Cases	Controls	P-value*		Cases	Controls	P-value*		Cases	Controls
F2-IsoP	(n = 56)	(n = 91)			(n = 77)	(n = 98)			(n = 133)	(n = 189)
Mean ± SD	0.32 ± 0.20	0.46 ± 0.38	0.01		0.36 ± 0.29	0.45 ± 0.31	0.0081		0.34 ± 0.26	0.45 ± 0.34
Median	0.28	0.37			0.26	0.34			0.28	0.36
Minimum	0.08	0.07			0.05	0.10			0.05	0.07
Maximum	0.91	1.83			1.44	1.61			1.44	1.83
Interquartile Range	0.21	0.33			0.34	0.31			0.29	0.32
PGF_{2α}	(n = 56)	(n = 91)			(n = 77)	(n = 98)			(n = 133)	(n = 189)
Mean ± SD	11.39 ± 14.13	6.93 ± 8.19	0.34		11.31 ± 13.55	6.35 ± 7.92	0.004		11.34 ± 13.74	6.63 ± 8.04
Median	4.78	2.87			7.92	2.78			7.00	2.81
Minimum	0.11	0.21			0.21	0.14			0.11	0.14
Maximum	51.73	34.17			79.17	39.47			79.17	39.47
Interquartile Range	14.80	9.86			15.53	10.51			15.84	10.28
PGE₂	(n = 53)	(n = 91)			(n = 66)	(n = 98)			(n = 119)	(n = 189)
Mean ± SD	6.00 ± 8.19	7.22 ± 9.83	0.08		3.04 ± 4.02	7.18 ± 8.07	<0.0001		4.36 ± 6.38	7.20 ± 8.94
Median	2.70	3.60			1.52	3.99			1.60	3.94
Minimum	0.01	0.11			0.02	0.05			0.01	0.05
Maximum	31.12	69.89			22.71	36.87			31.12	69.89
Interquartile Range	6.66	7.96			4.25	8.59			5.45	8.01
6-KPGF1α	(n = 43)	(n = 78)			(n = 49)	(n = 93)			(n = 92)	(n = 171)
Mean ± SD	0.42 ± 0.53	0.66 ± 1.07	0.11		0.41 ± 0.47	0.55 ± 0.87	0.30		0.41 ± 0.49	0.60 ± 0.97
										0.07

Parameters	African Americans				Caucasians				Total	
	Cases	Controls	P-value*		Cases	Controls	P-value*		Cases	Controls
Median	0.27	0.29			0.24	0.25			0.25	0.26
Minimum	0.00	0.00			0.01	0.00			0.00	0.00
Maximum	2.81	6.94			2.16	5.91			2.81	6.94
Interquartile Range	0.49	0.55			0.48	0.61			0.48	0.61
TxB2	(n = 51)	(n = 82)		(n = 63)	(n = 97)	(n = 114)		(n = 179)		
Mean ± SD	5.57 ± 7.60	4.34 ± 5.25	0.70	5.05 ± 12.91	5.15 ± 9.40	5.28 ± 10.82	0.43	4.78 ± 7.77	5.28 ± 10.82	4.78 ± 7.77
Median	1.60	3.00		1.85	1.64	1.79		2.22	1.79	2.22
Minimum	0.15	0.06		0.02	0.04	0.02		0.04	0.02	0.04
Maximum	30.54	27.87		96.14	60.61	96.14		60.61	96.14	60.61
Interquartile Range	7.13	4.68		3.06	4.55	3.73		4.62	3.73	4.62
PGD₂	(n = 44)	(n = 73)		(n = 40)	(n = 93)	(n = 84)		(n = 166)		
Mean ± SD	1.72 ± 1.72	3.22 ± 4.74	0.01	1.31 ± 1.71	2.63 ± 3.37	1.53 ± 1.72	0.0005	2.89 ± 4.03	1.53 ± 1.72	2.89 ± 4.03
Median	0.97	1.42		0.71	1.56	0.84		1.50	0.84	1.50
Minimum	0.02	0.15		0.03	0.06	0.02		0.06	0.02	0.06
Maximum	7.00	24.79		7.60	15.28	7.60		24.79	7.60	24.79
Interquartile Range	2.86	2.78		1.31	2.15	1.95		2.36	1.95	2.36

* P-values are based on log-transformed concentrations

TABLE 3

Comparison of amniotic fluid concentrations of F2-IsoP, PGF_{2α}, PGE₂, 6-KPGF1α, TxB2 and PGD2 between Caucasians and African Americans among preterm and normal term deliveries.

Parameters	Cases			Controls		
	African Americans	Caucasians	P-value*	African Americans	Caucasians	P-value*
F2-IsoP	(n = 56)	(n = 77)		(n = 91)	(n = 98)	
Mean ± SD	0.32 ± 0.20	0.36 ± 0.29	0.86	0.46 ± 0.38	0.45 ± 0.31	0.85
Median	0.28	0.26		0.37	0.34	
Minimum	0.08	0.05		0.07	0.10	
Maximum	0.91	1.44		1.83	1.61	
Interquartile Range	0.21	0.34		0.33	0.31	
PGF_{2α}	(n = 56)	(n = 77)		(n = 91)	(n = 98)	
Mean ± SD	11.39 ± 14.13	11.31 ± 13.55	0.44	6.93 ± 8.19	6.35 ± 7.92	0.31
Median	4.78	2.87		2.78	7.92	
Minimum	0.11	0.21		0.14	0.21	
Maximum	79.17	34.17		39.47	51.73	
Interquartile Range	14.80	15.53		9.86	10.51	
PGE₂	(n = 53)	(n = 66)		(n = 91)	(n = 98)	
Mean ± SD	6.00 ± 8.19	3.04 ± 4.02	0.073	7.22 ± 9.83	7.18 ± 8.07	0.86
Median	2.70	1.52		3.60	3.99	
Minimum	0.01	0.02		0.11	0.05	
Maximum	31.12	22.71		69.89	36.87	
Interquartile Range	6.66	4.25		7.96	8.59	
6-KPGF1α	(n = 49)	(n = 78)		(n = 93)	(n = 43)	
Mean ± SD	0.42 ± 0.53	0.40 ± 0.47	0.95	0.66 ± 1.07	0.55 ± 0.87	0.45
Median	0.27	0.24		0.29	0.25	

Parameters	Cases			Controls		
	African Americans	Caucasians	P-value*	African Americans	Caucasians	P-value*
Minimum	0.00	0.01		0.00	0.00	
Maximum	2.81	2.16		6.94	5.91	
Interquartile Range	0.49	0.48		0.55	0.61	
TXB2	(n = 51)	(n = 63)		(n = 82)	(n = 97)	
Mean ± SD	5.57 ± 7.59	5.05 ± 12.91	0.19	4.34 ± 5.25	5.15 ± 9.40	0.70
Median	1.60	1.85		3.00	1.64	
Minimum	0.15	0.02		0.06	0.04	
Maximum	30.54	96.14		27.87	60.61	
Interquartile Range	7.13	3.06		4.68	4.55	
PGD₂	(n = 44)	(n = 40)		(n = 73)	(n = 93)	
Mean ± SD	1.72 ± 1.72	1.31 ± 1.71	0.19	3.22 ± 4.74	2.63 ± 3.37	0.39
Median	0.97	0.71		1.42	1.56	
Minimum	0.02	0.03		0.15	0.06	
Maximum	7.00	7.60		24.79	15.28	
Interquartile Range	2.86	1.31		2.78	2.15	

* P-values are based on log-transformed concentrations

Comparison of amniotic fluid concentrations of F2-IsoP, PGF_{2α}, PGE₂, 6-KPGF1α, TxB2 and PGD2 between smokers and non-smokers among preterm and normal term

TABLE 4

Parameters	Cases		Controls	
	Smokers (n = 33)	Non-smokers (n = 100)	Smokers (n = 28)	Non-smokers (n = 161)
F2-IsoP				
Mean ± SD	0.60 ± 0.28	0.26 ± 0.19	0.92 ± 0.39	0.37 ± 0.26
Median	0.57	0.22	0.81	0.29
Minimum	0.12	0.05	0.31	0.07
Maximum	1.30	1.44	1.83	1.78
Interquartile Range	0.33	0.15	0.55	0.23
PGF_{2α}				
Mean ± SD	18.79 ± 16.76	8.88 ± 11.68	4.35 ± 5.46	7.03 ± 8.36
Median	13.45	4.14	1.17	3.16
Minimum	0.21	0.11	0.22	0.14
Maximum	79.17	64.00	22.73	39.47
Interquartile Range	17.98	11.83	6.84	11.18
PGE₂				
Mean ± SD	7.38 ± 8.12	3.25 ± 5.23	15.60 ± 14.98	5.74 ± 6.45
Median	5.49	1.30	14.21	3.59
Minimum	0.03	0.01	0.11	0.05
Maximum	31.12	30.52	69.89	29.72
Interquartile Range	6.90	3.52	21.36	6.69
6-KPGF1α				
Mean ± SD	0.45 ± 0.43	0.40 ± 0.51	1.05 ± 1.46	0.52 ± 0.83
Median	0.26	0.23	0.43	0.23
Minimum	0.08	0.00	0.16	0.00

Parameters	Cases			Controls		
	Smokers	Non-smokers	P-value*	Smokers	Non-smokers	P-value*
Maximum	1.62	2.81		6.94	5.91	
Interquartile Range	0.55	0.48		1.18	0.53	
TxB2	(n = 26)	(n = 88)		(n = 27)	(n = 152)	
Mean ± SD	7.89 ± 18.70	4.51 ± 6.99	0.14	7.89 ± 9.52	4.23 ± 7.31	0.001
Median	2.75	1.57		4.77	1.68	
Minimum	0.12	0.02		0.32	0.04	
Maximum	96.14	31.75		40.63	60.61	
Interquartile Range	3.11	3.61		7.28	4.43	
PGD₂	(n = 19)	(n = 65)		(n = 26)	(n = 140)	
Mean ± SD	2.18 ± 1.99	1.33 ± 1.60	0.06	5.40 ± 5.14	2.42 ± 3.62	<0.0001
Median	1.42	0.69		3.42	1.23	
Minimum	0.05	0.02		0.38	0.06	
Maximum	7.00	7.60		22.66	24.79	
Interquartile Range	3.24	1.42		5.71	1.74	

* P-values are based on the log-transformed concentrations

TABLE 5

Racial comparison of amniotic fluid concentrations of F2-IsoP, PGF_{2α}, PGE₂, 6-KPGF1α, TxB2 and PGD2 between smokers and non-smokers among preterm and normal term deliveries stratified by race.

Parameters	African Americans						Caucasians					
	Cases			Controls			Cases			Controls		
	Smokers (n = 13)	Non-Smokers (n = 30)	P- value*	Smokers (n = 12)	Non-Smokers (n = 79)	P- value*	Smokers (n = 19)	Non-Smokers (n = 58)	P- value*	Smokers (n = 16)	Non-Smokers (n = 82)	P- value*
F2-IsoP												
Mean ± SD	0.55 ± 0.17	0.24 ± 0.14	<0.0001	0.88 ± 0.47	0.39 ± 0.31	<0.0001	0.63 ± 0.34	0.27 ± 0.22	<0.0001	0.95 ± 0.33	0.35 ± 0.19	<0.0001
Median	0.54	0.22		0.75	0.33		0.60	0.22		0.99	0.28	
Minimum	0.31	0.08		0.31	0.07		0.12	0.05		0.44	0.10	
Maximum	0.88	0.91		1.83	1.78		1.30	1.44		1.61	0.99	
Interquartile Range	0.28	0.15		0.48	0.24		0.44	0.22		0.55	0.21	
PGF_{2α}												
Mean ± SD	20.56 ± 15.37	8.33 ± 12.43	<0.0001	4.32 ± 6.44	7.32 ± 8.39	0.18	17.48 ± 18.01	9.29 ± 11.20	0.02	4.37 ± 4.82	6.74 ± 8.36	0.64
Median	18.99	2.07		1.15	3.49		13.05	7.06		1.27	3.14	
Minimum	4.14	0.11		0.22	0.21		0.21	0.24		0.26	0.14	
Maximum	51.73	49.57		22.73	34.17		79.17	64.00		13.72	39.47	
Interquartile Range	26.64	11.22		5.57	10.97		17.26	14.85		7.87	11.54	
PGE₂												
Mean ± SD	11.13 ± 9.00	4.16 ± 7.13	<0.0001	16.90 ± 19.85	5.75 ± 6.28	0.04	4.45 ± 6.14	2.51 ± 2.77	0.48	14.62 ± 10.63	5.73 ± 6.64	0.002
Median	7.35	1.29		10.02	3.44		2.17	1.34		15.65	3.74	
Minimum	1.36	0.01		0.11	0.14		0.03	0.02		1.03	0.05	
Maximum	31.12	30.52		69.89	28.66		22.71	9.70		36.87	29.72	
Interquartile Range	9.43	4.44		23.90	7.22		5.43	3.55		16.62	6.07	
6-KPGF1α												
Mean ± SD	0.52 ± 0.49	0.38 ± 0.54	0.09	1.38 ± 1.98	0.54 ± 0.81	0.01	0.37 ± 0.36	0.42 ± 0.50	0.47	0.81 ± 0.96	0.49 ± 0.84	0.01

Parameters	African Americans						Caucasians					
	Cases			Controls			Cases			Controls		
	Smokers	Non-Smokers	P-value*	Smokers	Non-Smokers	P-value*	Smokers	Non-Smokers	P-value*	Smokers	Non-Smokers	P-value*
Median	0.29	0.20		0.43	0.25		0.22	0.26		0.43	0.20	
Minimum	0.08	0.00		0.20	0.00		0.10	0.01		0.16	0.00	
Maximum	1.62	2.81		6.94	3.96		1.13	2.16		3.93	5.91	
Interquartile Range	0.57	0.48		1.60	0.55		0.53	0.50		0.76	0.51	
TxB2	(n = 13)	(n = 38)		(n = 11)	(n = 71)		(n = 13)	(n = 50)		(n = 16)	(n = 81)	
Mean ± SD	5.43 ± 5.18	5.62 ± 8.32	0.20	7.26 ± 7.77	3.89 ± 4.66	0.07	10.35 ± 26.24	3.67 ± 5.73	0.44	8.32 ± 10.78	4.53 ± 9.04	0.006
Median	3.14	1.37		4.59	2.86		1.77	1.96		5.07	1.38	
Minimum	0.67	0.15		0.32	0.06		0.12	0.02		0.81	0.04	
Maximum	16.81	30.54		26.55	27.87		96.14	31.75		40.63	60.61	
Interquartile Range	6.18	7.19		8.43	4.62		1.87	3.14		5.73	3.51	
PGD₂	(n = 11)	(n = 33)		(n = 10)	(n = 63)		(n = 8)	(n = 32)		(n = 16)	(n = 77)	
Mean ± SD	3.31 ± 1.91	1.19 ± 1.30	0.002	6.17 ± 6.66	2.75 ± 4.25	0.02	0.63 ± 0.46	1.48 ± 1.86	0.57	4.92 ± 4.10	2.15 ± 3.02	0.0007
Median	3.49	0.57		3.51	1.28		0.55	0.78		3.10	1.13	
Minimum	0.30	0.02		0.39	0.15		0.05	0.03		0.38	0.06	
Maximum	7.00	4.59		22.66	24.79		1.42	7.60		15.28	14.40	
Interquartile Range	2.6	1.26		5.61	2.14		0.66	1.58		6.14	1.53	

* P-values are based on log-transformed concentrations

TABLE 6

Preterm Birth comparison of amniotic fluid concentrations of F2-IsoP, PGF_{2α}, PGE₂, 6-KPGF1α, TxB2 and PGD₂ between cases with infections and no infection.

Parameters	Cases		P-value *
	Infection	No Infection	
F2-IsoP **	(n=29)	(n=95)	
Mean ± SD	0.45 ± 0.35	0.31 ± 0.22	0.02
Median	0.33	0.24	
Minimum	0.09	0.05	
Maximum	1.44	1.30	
Interquartile Range	0.37	0.25	
PGF_{2α}	(n=29)	(n=95)	
Mean ± SD	14.83 ± 14.39	11.40 ± 11.26	0.08
Median	6.49	7.40	
Minimum	0.11	0.14	
Maximum	51.73	79.17	
Interquartile Range	13.44	17.73	
PGE₂	(n=28)	(n=84)	
Mean ± SD	4.09 ± 3.95	4.66 ± 7.21	0.43
Median	3.13	1.41	
Minimum	0.03	0.01	
Maximum	16.71	31.12	
Interquartile Range	5.24	5.54	
6-KPGF1α	(n=19)	(n=67)	
Mean ± SD	0.57 ± 0.72	0.38 ± 0.42	0.47
Median	0.26	0.25	
Minimum	0.01	0.002	
Maximum	2.81	2.16	
Interquartile Range	0.83	0.50	
Thromboxane: TxB2	(n=25)	(n=82)	
Mean ± SD	4.52 ± 5.30	5.88 ± 12.36	0.93
Median	2.55	1.83	
Minimum	0.07	0.09	
Maximum	19.01	96.14	
Interquartile Range	5.76	3.78	
PGD₂	(n=19)	(N=58)	
Mean ± SD	1.57 ± 1.64	1.62 ± 1.82	0.35

Parameters	Cases		P-value *
	Infection	No Infection	
Median	0.88	0.85	
Minimum	0.21	0.02	
Maximum	5.58	7.60	
Interquartile Range	2.15	2.88	

* P-values are based on log-transformed concentrations

** Data on infection status were missing in a total of 9 cases.

Preterm Birth comparison of amniotic fluid concentrations of F2-IsoP, PGF_{2α}, PGE₂, 6-KPGF1α, TxB2 and PGD2 between women with infections and no infection among African Americans and Caucasians

TABLE 7

Parameters	African Americans			Caucasians		
	Infection	No Infection	P-value *	Infection	No Infection	P-value *
F2-IsoP	(n=10)	(n=42)		(n=19)	(n=53)	
Mean ± SD	0.32 ± 0.18	0.32 ± 0.21	0.92	0.52 ± 0.39	0.30 ± 0.23	0.008
Median	0.30	0.28		0.36	0.23	
Minimum	0.09	0.08		0.11	0.05	
Maximum	0.62	0.91		1.44	1.30	
Interquartile Range	0.26	0.21		0.57	0.27	
PGF_{2α}	(n=10)	(n=42)		(n=19)	(n=53)	
Mean ± SD	14.28 ± 18.40	11.52 ± 13.54	0.88	12.07 ± 15.14	9.88 ± 12.07	0.65
Median	5.80	4.45		6.60	7.92	
Minimum	0.11	0.14		0.34	0.21	
Maximum	51.73	49.57		36.49	79.17	
Interquartile Range	15.19	21.26		13.44	16.49	
PGE₂	(n=9)	(n=41)		(n=19)	(n=43)	
Mean ± SD	3.83 ± 2.53	6.76 ± 9.11	0.63	4.21 ± 4.52	2.65 ± 3.89	0.33
Median	3.88	1.41		2.88	1.37	
Minimum	0.43	0.01		0.03	0.02	
Maximum	7.55	31.12		16.71	22.71	
Interquartile Range	4.54	8.31		7.03	3.51	
6-KPGF1α	(n=5)	(n=34)		(n=14)	(n=33)	
Mean ± SD	0.82 ± 1.12	0.39 ± 0.40	0.23	0.48 ± 0.55	0.38 ± 0.45	0.93
Median	0.46	0.28		0.21	0.25	
Minimum	0.10	0.002		0.01	0.01	

Parameters	African Americans			Caucasians		
	Infection	No Infection	P-value*	Infection	No Infection	P-value*
Maximum	2.81	1.62		1.56	2.16	
Interquartile Range	0.21	0.50		0.83	0.49	
Thromboxane: TxB2						
	(n=8)	(n=39)		(n=17)	(n=43)	
Mean ± SD	6.07 ± 6.36	5.89 ± 8.15	0.64	3.79 ± 4.76	5.86 ± 15.32	0.97
Median	3.41	1.60		2.48	1.87	
Minimum	0.22	0.15		0.07	0.09	
Maximum	17.10	30.54		19.01	96.14	
Interquartile Range	9.68	8.43		4.09	3.06	
PGD₂						
	(n=6)	(n=34)		(n=13)	(n=24)	
Mean ± SD	1.50 ± 1.62	1.91 ± 1.80	0.94	1.60 ± 1.71	1.22 ± 1.80	0.09
Median	0.50	1.38		1.07	0.50	
Minimum	0.39	0.02		0.21	0.03	
Maximum	3.68	7.00		5.58	7.60	
Interquartile Range	3.07	2.84		0.68	1.40	

* P-values are based on log-transformed concentrations