

Ambient exposure to fine particulate matter with oxidative potential affects oxidative stress biomarkers in pregnancy

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Abstract

Prenatal exposures to ambient particulate matter (PM_{2.5}) from traffic may generate oxidative stress and thus contribute to adverse birth outcomes. We investigated whether PM_{2.5} constituents from brake and tire wear affect levels of oxidative stress biomarkers (malondialdehyde [MDA], 8-hydroxy-2'-deoxyguanosine [8-OHdG]) using urine samples collected up to 3 times during pregnancy in 156 women recruited from antenatal clinics at the University of California Los Angeles. Land use regression models with co-kriging were employed to estimate average residential outdoor concentrations of black carbon (BC), PM_{2.5} mass, PM_{2.5} metal components, and 3 PM_{2.5} oxidative potential metrics during the 4 weeks prior to urine sample collection. The 8-OHdG concentrations in mid-pregnancy increased by 24.8% (95% confidence interval [CI], 9.0–42.8) and 14.3% (95% CI, 0.4%–30.0%) per interquartile range (IQR) increase in PM_{2.5} mass and BC, respectively. The brake wear marker (barium) and the oxidative potential metrics were associated with increased MDA concentration in the first sample collected (10–17 gestational week), but 95% CIs included the null. Traffic-related air pollution contributed in early to mid-pregnancy to oxidative stress generation previously linked to adverse birth outcomes.

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Key words: traffic related air pollution; fine particulate matter; oxidative stress; pregnancy.

Introduction

Prenatal exposure to fine particulate matter (PM_{2.5}), especially from traffic-related sources, may affect pregnancy by inducing oxidative stress, which leads to adverse birth outcomes.^{1–3} Non-exhaust PM_{2.5} emissions from brake wear, tire wear, and road wear particles are currently estimated to contribute up to 85% of primary PM_{2.5} emissions from road traffic, yet their contributions are scarcely addressed by regulation and technology development.⁴ Previous studies have identified specific metals as tracers for brake wear (barium or copper) and zinc for tire wear particles.^{5,6} Furthermore, metal components in PM_{2.5} are also capable of inducing the production of reactive oxygen species (ROSs) and contributing to an increased level of oxidative stress and a pro-inflammatory state in the lung.⁷ Human studies have employed multiple biomarkers to reflect the oxidative status of participants including 8-hydroxy-2'-deoxyguanosine (8-OHdG), which represents one of the most abundant, sensitive, and extensively studied

free radical-induced DNA lesions,^{8,9} and malondialdehyde (MDA), a frequently measured oxidative stress biomarker that represents lipid peroxidation products.¹⁰

Epidemiological studies found that short-term PM exposures (averaged over 1–12 days prior to sample collection) were associated with increased concentrations of these oxidative stress biomarkers, measured in the blood or urine of healthy young adults, children, or the elderly.^{11–16} To date, only 1 study investigated the effects of modeled PM_{2.5} on urinary oxidative stress biomarker levels in pregnant women,¹⁷ and none evaluated traffic related metals from brake and tire wear in an environment with relatively low levels of particulate matter.

Combined with an extensive special traffic pollutant monitoring program, we employed land use regression (LUR) modeling with co-kriging to estimate PM_{2.5} mass, PM_{2.5} metal constituents from brake wear (using barium as a marker) and tire wear (using zinc as a marker), as well as the oxidative potential matrix of

the particulate mixture. We evaluated associations between these modeled 4-week average (prior to sample collection) outdoor air pollutant and oxidative potential concentrations with oxidative stress biomarkers measured in urine samples collected multiple times during pregnancy from pregnant women enrolled at University of California Los Angeles (UCLA) antenatal clinics.

Methods

Study population

The Imaging Innovations for Placental Assessment in Response to Environmental Exposures (PARENTs) study assembled a cohort of pregnant women in Los Angeles with the purpose of developing and testing novel magnetic resonance imaging (MRI) tools for the early recognition of placental insufficiency.¹⁸ The PARENTs cohort recruited 199 pregnant women early in their pregnancies from antenatal clinics at UCLA during 2016–2019. Women were asked to participate in a once-per-trimester and at delivery study visit that included urine sample collection for biomarkers assessment. Phone interviews were conducted at 3 time points during pregnancy and at birth to collect environmental and behavioral risk factor data.

Our study population consisted of 156 women enrolled in the PARENTs study for whom at least 1 useable urine sample and geocodable residential address were available within the boundaries of multiple exposure surfaces we had generated. The research protocol for this study was approved by the UCLA Institutional Review Board.

Exposure estimation

Sample collection and measurement

We focused on modeling PM_{2.5} mass and a total of 6 speciated PM_{2.5} components including 2 PM_{2.5} metals (barium and zinc as tracers for brake and tire wear, respectively); black carbon (BC) representing the vehicle exhaust emission from incomplete combustion of fossil fuels, especially diesels; 2 measures of PM_{2.5} oxidative potential with assays of OH formation in simulated lung fluid (OP^{OH}) and loss of dithiothreitol in phosphate buffer (OP^{DTT}); a 2-hour modeled ROS measure (ROS) based on measured concentrations of PM_{2.5} iron and PM_{2.5} copper,¹⁹ both associated with brake and tire wear and active in oxidative potential assays.²⁰

Details of PM_{2.5} sample collection, measurements, and modeling methods are described elsewhere.^{5,6,19,20} Briefly, PM_{2.5} samples were collected on pre-cleaned Teflon membrane filters with Harvard cascade impactors²¹ and PM_{2.5} impactors (H-PEM, BGI Inc.) in 2 seasons (September 2019 and February 2020). In each season, samples were collected simultaneously for 2-weeks at a total of 46 different outdoor locations that were comprised of a mix of individual homes of study participants independent of the outcome and government regulatory air monitoring stations in the Greater Los Angeles region.²² Four sites were measured in both seasons, creating a total of 50 samples.

The Harvard cascade impactor filter samples were analyzed for PM mass and for metal and element speciation, including barium, zinc, iron, and copper, via Sector Field Inductively Coupled Plasma Mass Spectrometry (SF-ICP-MS).⁵ The modeled ROS concentrations were estimated based on the measured PM_{2.5} constituents copper and iron from SF-ICP-MS using a kinetic multi-layer model of surface and bulk chemistry in the epithelial lining fluid (KM-SUB-ELF), which is based on a series of mathematical formulas to describe mass transport and chemical reactions involving redox-active pollutants, antioxidants, and surfactants in the lung.¹⁹ Furthermore, the PM_{2.5} impactor samples were

analyzed for BC concentrations and oxidative potential including hydroxyl radical formation (OP^{OH}) and dithiothreitol loss (OP^{DTT}) in acellular assays performed at our UCLA lab.²⁰ See more details in Appendix S1.

Exposure modeling

The monthly spatiotemporal concentrations of PM_{2.5} mass and 6 speciated PM_{2.5} components were modeled at a resolution of 30 by 30 meters with co-kriging with a land use regression model as an external drift.⁶ The external drift in each co-kriging model, a linear combination of predictors, was calculated based on our measurements and land use data including the 2016 National Land Cover Database,²³ 2018 traffic volume,²⁴ road network and rail network information,²⁵ and location of brake and tire wear-related businesses, such as auto repair shops.²⁶ We also obtained hourly averaged PM_{2.5} concentration data between March 2019 and February 2020 (overlapping with our filter collection periods and avoiding major traffic disruption periods associated with the COVID-19 pandemic) from 143 PurpleAir sensors (<https://www2.purpleair.com/>) distributed across the Greater Los Angeles region that provided publicly available monitoring data. These data were used as a secondary or auxiliary variable in the co-kriging models. The model performance was presented in Table S1 (see Liu et al 2022⁶ for details).

Exposure assessment

After geocoding all maternal addresses of residence throughout pregnancy obtained in phone interviews, we estimated each mother's exposures to PM_{2.5} mass, BC, metals as markers of brake and tire wear (barium, zinc), measures of oxidative potential (OP^{OH} and OP^{DTT}), and ROS by overlaying the co-kriging surfaces onto the geocoded addresses. The PM_{2.5} mass was predicted as $\mu\text{g}/\text{m}^3$; metals, OP^{OH}, and OP^{DTT} were predicted as normalized concentrations or activities according to PM_{2.5} mass per volume of air sampled (ng/m^3 for barium and zinc, $\text{pmol}/\text{min}/\text{m}^3$ for OH formation and DTT loss). Black carbon was predicted as a percentage of PM_{2.5} mass; ROSs were treated as continuous measures in nmol/L .

Our study mainly focused on long-term spatial patterns that are expected to be maintained despite some year-to-year changes in land use, meteorology, and other factors.^{27,28} To obtain monthly estimates of co-kriged speciated PM_{2.5} exposure and PM_{2.5} mass, we first calculated monthly averages for PurpleAir hourly monitoring data. Then, for each month, the cross-covariance between each of the speciated PM_{2.5} exposures/PM_{2.5} mass and PurpleAir PM_{2.5} averages were modeled and used to predict monthly average exposure estimates using the *gstat* R package that allowed us to implement co-kriging and generate predicted monthly average exposure estimates. As each participant was scheduled for study visits spaced by at least a 4-week interval, we selected a 4-week period prior to urine sample collection to obtain mutually exclusive exposure averaging periods for each sample. We calculated the weighted average (by days in each month a participant resided at the address) for each exposure during the 4-week period prior to each urine sample collection date.

Oxidative stress biomarkers assessment

Maternal urine samples were obtained from mothers at each study visit at least once and at most 3 times during pregnancy, with the first sample collection in the 10th–17th gestational week, the second sample collection in the 18th–29th gestational week, and the third sample collection after the 30th gestational week and prior to delivery. The first 2 visits were timed to be optimal for MRI evaluations of the placental blood flow.

The urine samples were stored at -80°C after collection at UCLA and were shipped on dry ice to the Emory Children's Health Exposure Analysis Resource (CHEAR) Laboratory Hub to measure the oxidative stress biomarkers malondialdehyde (MDA) and 8-hydroxy-2'-deoxyguanosine (8-OHdG). All samples were randomized using a Fisher-Yates shuffling algorithm prior to analysis to reduce any potential batch effects.^{29,30} Malondialdehyde and 8-OHdG concentrations in urine samples were measured by liquid chromatography-mass spectrometry (LC-MS).³¹ Samples with measures below the limit of detection (LOD) values were replaced with the LOD/sqrt2.³² Oxidative stress biomarker concentrations were further corrected for urine dilution by adjusting for specific gravity (SG) measured with a Reichert AR200 refractometer. We excluded samples with an invalid SG value below 1 ($n = 14, 18$, and 16 samples during the first, second, and third sample collection interval, respectively), resulting in a total of 382 samples for analysis. To correct for the hydration status of the pregnant women, the SG-standardized biomarker concentrations were calculated using the following formula³³:

$$\text{CHEM}_{\text{SG-Adj}} = \text{CHEM}_i * [(SG_m - 1) / (SG_i - 1)]$$

where $\text{CHEM}_{\text{SG-Adj}}$ is the specific gravity-standardized biomarker concentration (nmol/L for MDA and ng/mL for 8-OHdG), CHEM_i is the observed biomarker concentration, SG_i is the specific gravity of the urine sample, and SG_m is the median specific gravity for the total samples with valid SG values.

Covariates

In interviews, we collected data on potential confounding variables including maternal age (years), parity (continuous), maternal pre-pregnancy body mass index (BMI; <18.5 , 18.5 - 24.9 , 25 - 30 , and >30), maternal race/ethnicity (White, non-White), maternal educational attainment (bachelor's degree or less, master's degree, doctoral/professional degree), and maternal employment status (employed/student, unemployed). We also obtained gestational age based on the best obstetric estimate from the first trimester ultrasound, as well as information about pregnancy complications including gestational diabetes, gestational hypertension and pre-eclampsia from medical records. Season of conception was categorized based on the month of conception as in Table 1.

Statistical analysis

We scaled each pollutant concentration according to its interquartile range (IQR) in all samples to allow for comparison across pollutants. Oxidative stress biomarker concentrations were treated as continuous variables and log-transformed for statistical analyses.

To generate effect estimates in different time intervals during pregnancy, we first conducted multiple linear regression analyses to estimate effects between $\text{PM}_{2.5}$ mass/speciated $\text{PM}_{2.5}$ components (metals and oxidative potential metrics) and concentrations of oxidative stress biomarkers during each urine sample collection interval, separately. Subsequently, we used a linear mixed model with a random intercept for participant to take repeated measurements into account (ie, we relied on data for up to 3 time points across pregnancy for each exposure and biomarker and assessed longitudinal associations between $\text{PM}_{2.5}$ mass/speciated $\text{PM}_{2.5}$ components and oxidative stress urine measures). In simple one point in time linear and longitudinal linear mixed regression models, we adjusted for maternal age, race/ethnicity, education,

Table 1. Characteristics of the study population ($n = 156$)

Characteristics	N	%
Maternal age, years		
≤24	2	1.3
25-29	21	13.5
30-34	76	48.7
≥35	57	36.5
Parity		
0	73	46.8
1	64	41.0
>1	19	12.2
Maternal race		
Asian or Pacific islander	44	28.2
Black, non-Hispanic	10	6.4
Hispanic	28	18.0
White, non-Hispanic	73	46.8
American Indian or Alaskan Native	1	0.6
Maternal education		
High school graduate	1	0.7
Some College	7	4.6
Vocational, technical, associates or other	8	5.3
2-year degree		
Bachelor's degree	53	35.0
Master's degree	45	29.6
Doctoral degree or professional degree	38	25.0
Missing	4	
Employment Status		
Employed or student	137	90.1
Not employed	15	9.9
Missing	4	
Pre-pregnancy BMI		
Underweight	5	3.2
Normal	101	64.7
Overweight	32	20.5
Obese	18	11.5
Gestational diabetes		
Yes	20	12.8
No	136	87.2
Gestational hypertension		
Yes	15	9.6
No	141	90.4
Pre-eclampsia		
Yes	16	10.3
No	140	89.7
Season of conception		
Spring (March, April, May)	33	21.2
Summer (June, July, August)	41	26.3
Fall (September, October, November)	36	23.1
Winter (December, January, February)	46	29.5

pre-pregnancy BMI, and season of conception. Sensitivity analyses were conducted by additionally adjusting for maternal employment status, parity, and gestational days at sample collection. Furthermore, considering the likely higher oxidative stress among women experiencing pregnancy complications,³⁴ we restricted analyses to women without pregnancy complications related to higher oxidative stress states (gestational diabetes, gestational hypertension, pre-eclampsia). All statistical analyses were performed using SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

Results

Most participants were ≥ 30 years old, had achieved high levels of education, and were employed. More than half of the mothers were parous, and almost a third (30%) were overweight or obese

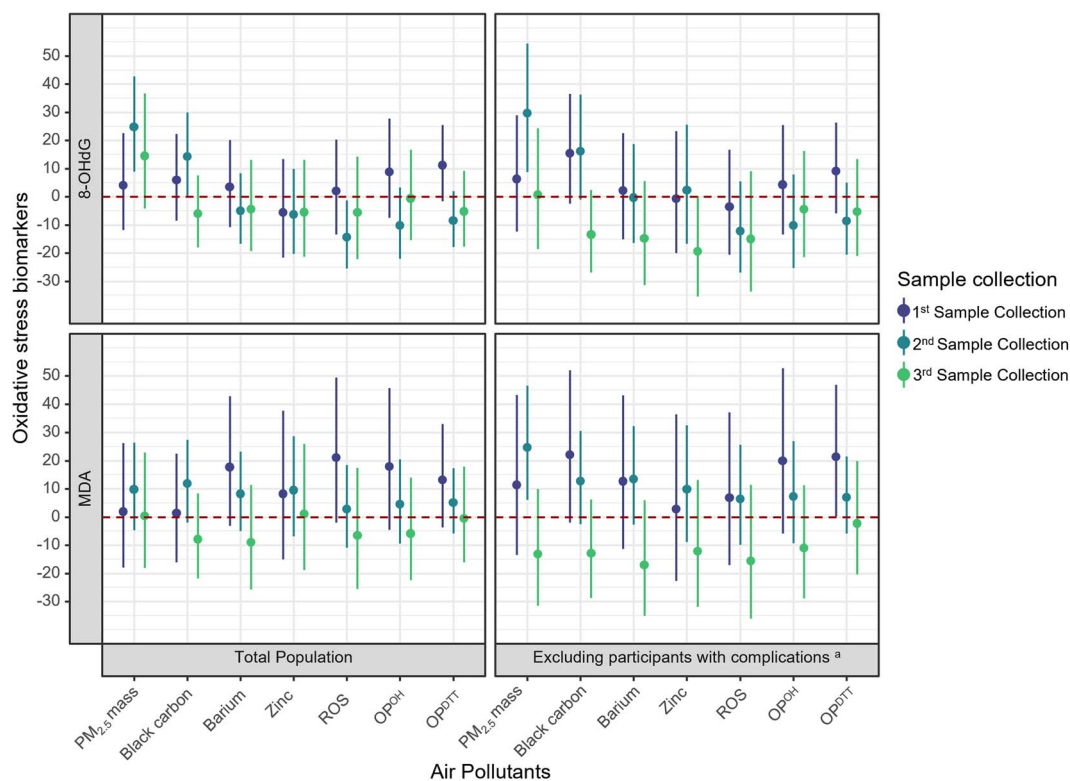


Figure 1. Linear regression models for percentage change and 95% confidence interval (CI) in concentration of oxidative stress biomarker per interquartile range (IQR) increase of time-window specific exposure to each air pollutant. Adjusted for maternal age, race/ethnicity, education, pre-pregnancy BMI, season of conception. a. Excluded participants with gestational diabetes, gestational hypertension, or pre-eclampsia during pregnancy ($n = 47$).

(BMI ≥ 25 ; Table 1). Approximately half of the mothers reported their race/ethnicity as non-Hispanic White; 18% as Hispanic; and 28% as Asian/Pacific Islander.

SG adjusted 8-OHdG mean concentrations decreased from the first to the third gestational sampling period, while the mean MDA concentration decreased from the first to the second sample period but then increased between the second to the third (Table S2). Specific gravity adjusted oxidative stress biomarkers varied slightly by sample collection season (Table S3), with the lowest mean values observed in summer for both MDA and 8-OHdG. The average levels for PM_{2.5} mass/speciated PM_{2.5} exposures were similar across gestational sample collection periods (Table S4). Pearson correlations between all pollutants were also similar in all 3 periods (Figure S1).

Concentration of 8-OHdG (DNA damage marker) increased during the second sample collection period by 24.8% (95% confidence interval [CI], 9.0%-42.8%) per IQR increase in PM_{2.5} mass averaged over the 4 weeks prior to sample collection (Figure 1; Table S5). A similar but weaker association was observed for BC (ie per IQR increase 8-OHdG increased by 14.3%; 95% CI, 0.4%-30.0%) during the second sample collection period. Most of the tracer metals for brake and tire wear and oxidative potential metrics were not associated with an increase in 8-OHdG levels, except for OP^{DTT} with a 11.2% increase at the first sample collection time, but the 95% CI included the null (95% CI, -1.6% to 25.6%). We found positive associations for MDA (lipid peroxidation marker) in the first sample collection period with brake wear (barium) and all oxidative potential metrics (ROS, OP^{OH}, OP^{DTT}), increasing MDA by 13.2% to 21.1%, but all 95% CIs included the null (Figure 1; Table S5). Particulate matter mass and black carbon were associated with urinary MDA increases in the

second sample collection period, although the 95% CIs included the null. Moreover, we observed no changes in MDA levels per IQR increase in PM_{2.5} metals or oxidative potential metrics during the second or third sample collection period (Figure 1; Table S5).

We did not observe associations between PM_{2.5} mass/speciated PM_{2.5} exposures and urinary concentrations of 8-OHdG or MDA in linear mixed models across all 3 gestational sampling periods. However, linear mixed models that included only the first and second sample collection period, showed results consistent with those from separate linear regression models. Specifically, we estimated a 15.6% (95% CI, 3.3%-29.4%) increase of 8-OHdG levels associated with an IQR increase in PM_{2.5} mass, and a 9.6% (95% CI, -1.2% to 21.5%) increase for BC with 8-OHdG (Figure 2; Table S6) but not for tracer metals for brake and tire wear or oxidative potential metrics. For all PM_{2.5} mass and speciated PM_{2.5} components (metals and oxidative potential metrics), we also saw increases in urinary MDA concentrations in linear mixed models including the first 2 periods, but 95% CIs crossed the null (Figure 2; Table S6).

After excluding women with pregnancy complications (gestational diabetes, gestational hypertension, or pre-eclampsia), most of the estimated associations were stable, with higher point estimates and wider 95% CIs (Table S5 and Table S6). Also, after these exclusions, associations for PM_{2.5} mass with MDA were much stronger and statistically significant in regression models for the second gestational sample collection interval (24.7%; 95% CI, 6.2%-46.6%) and in linear mixed models that included both the first and second sample collection periods (20.2%; 95% CI, 3.9%-39.0%). In sensitivity analyses additionally adjusting for parity, maternal employment status, and gestational day at sample collection, effect estimates from both linear or linear

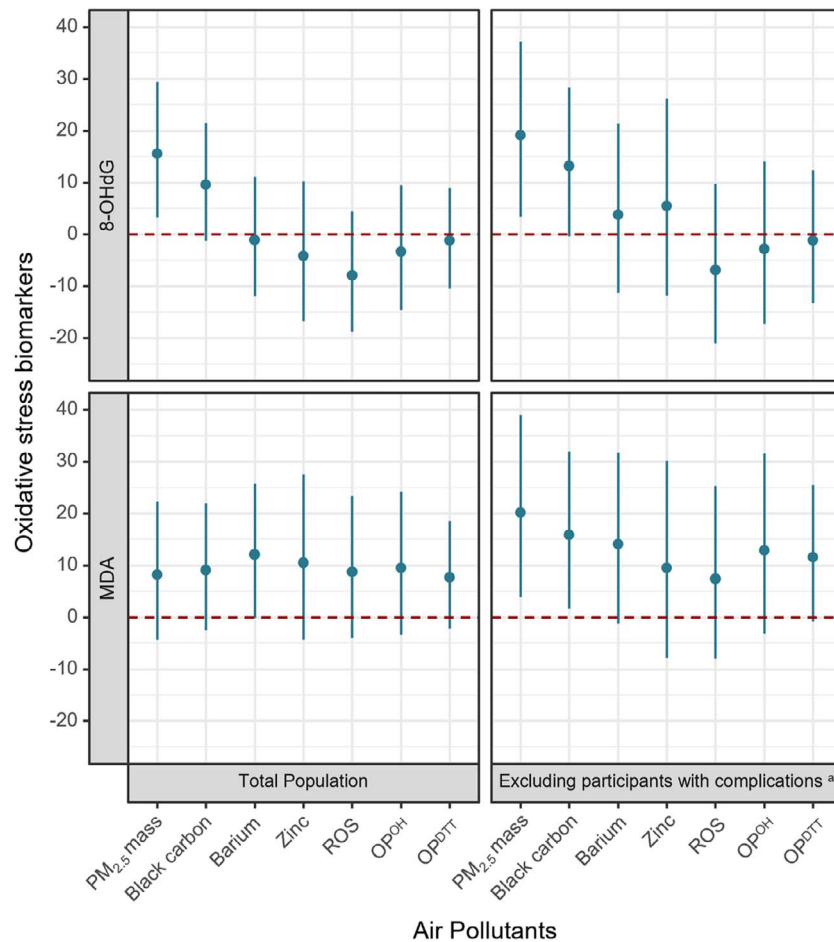


Figure 2. Linear mixed regression models for percentage change and 95% confidence interval (CI) in concentration of oxidative stress biomarker per interquartile range (IQR) increase of time-window specific exposure to each air pollutant. Adjusted for maternal age, race/ethnicity, education, pre-pregnancy BMI, and season of conception. a. Excluded participants with gestational diabetes, gestational hypertension, or pre-eclampsia during pregnancy ($n = 45$).

mixed regression models changed only minimally (Table S7 and Table S8).

Discussion

We found increases in urinary 8-OHdG for increases in medium-term exposure to $PM_{2.5}$ mass and BC in mid-pregnancy. $PM_{2.5}$ mass and BC were also associated with urinary MDA increases in mid-pregnancy among women with or without pregnancy complications, although some of the 95% CIs included the null. Urinary MDA levels were positively associated with the brake wear marker (barium) and with oxidative potential metrics (ROS, OP^{OH} and OP^{DTT}) in early to mid-pregnancy in women, although 95% CIs included the null.

A growing number of studies provide evidence that modern lifestyles such as exposure to pollutants, poor diet, and lack of exercise can cause oxidative stress, and ultimately DNA damage that increases the risks of infertility, miscarriage, and late-gestation pregnancy complications.³⁵ Previous studies that investigated effects of $PM_{2.5}$ mass/speciated $PM_{2.5}$ exposures on urinary oxidative stress biomarkers focused mainly on short-term exposures and targeted children or general adult populations.^{12,13} Our findings for medium-term (4-week average) exposures to $PM_{2.5}$ mass and BC and urinary measures of 8-OHdG and MDA in mid-pregnancy are consistent with these published reports.

A study of 36 school children collected hourly air pollution exposures and 5 urine samples during a 10-12 day period before and during the 2008 Beijing Olympic Games and reported reduced levels of both urinary measurements of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) and MDA with Olympic games related air pollution reductions including levels of BC and $PM_{2.5}$ mass.¹² A randomized crossover trial with 53 healthy nonsmoking volunteers found that urinary 8-OHdG increased by 14% after 1 hour of exposure to copper, a metal component of brake wear²⁰; increased levels of 8-OHdG and MDA were also associated with ascorbate- and glutathione-depletion measures of oxidative potential 21 hours after exposure.¹³

We did not observe associations between urinary 8-OHdG levels and our modeled levels of heavy metals as markers of brake and tire wear. Heavy metal-related DNA damage in early pregnancy, however, may impede fetal survival if not repaired immediately, as serious disruptions of the balance between ROS and antioxidants during pregnancy that result in DNA damage can lead to early pregnancy loss.³⁵ Thus, null effects for heavy metals may be due to fetal survivor or "live birth" bias.³⁶ Specifically, our study required participants to have had a live birth. Pregnant women most highly exposed to toxics from brake and tire wear would be expected to exhibit higher levels of 8-OHdG, but they also may experience fetal loss and thus would not be included in our study. Furthermore, more than 80% of the participants were >30 years

old at time of pregnancy. Baseline DNA damage and pregnancy failure rises with age,³⁷ which may explain why the highest exposures may have contributed to such loss.

Few studies previously examined associations between PM_{2.5} constituents and oxidative stress levels in pregnant women. One study monitored PM_{2.5} during a 7 day period before delivery and measured urinary 8-oxodG in mothers and newborns living in 2 locations with differing levels of air pollution.³⁸ Although they found higher levels of 8-oxodG in newborns in the location with higher PM_{2.5} exposure levels, PM_{2.5} exposures were not related to urinary 8-oxodG in pregnant women at the time of delivery. Similarly, in our study, late pregnancy urinary 8-OHdG levels did not increase with PM exposures, possibly reflecting the difficulty in assessing impacts on this biomarker during a pregnancy phase with physiologically high oxidative stress or a higher efficiency of DNA damage repair in mothers with a successful pregnancy. Another study in maternal and cord blood reported that PM_{2.5} exposure increased mitochondrial 8-OHdG levels in maternal blood during the entire pregnancy especially in the third trimester.³⁹ They also found associations between maternal particulate air pollution and newborn mitochondrial genome (mtDNA) damage most strongly associated with the first and second trimester exposure window, suggesting that the susceptible windows for mtDNA oxidative damage to the fetus or placenta may be in early to mid-pregnancy, which are also critical exposure windows for some adverse birth outcomes including pre-eclampsia and preterm birth.^{40–42} In addition, urinary 8-OHdG reflects nuclear genome DNA damage that can be repaired while repair mechanisms for damage to the mtDNA are less efficient and mtDNA damage may therefore be more persistent.^{39,43}

In terms of lipid peroxidation measures, our study estimated positive effects that were not formally statistically significant for most pollutants unless we excluded women who experienced pregnancy complications. This again argues to the difficulty in assessing air pollution influences on oxidative stress biomarkers when physiological levels of oxidative stress are already high, such as due to pregnancy complications that manifest in mid-pregnancy and are known to affect this pathway.³⁴ This would mask possible air pollution-related effects. A previous study reported that serum levels of MDA increased with each IQR increase in LUR-modeled ambient PM_{2.5} concentrations over the preceding 8 days in early pregnancy, suggesting exposure to ambient PM_{2.5} may induce systemic lipid peroxidation in early pregnancy in women.¹⁷ In contrast, we observed associations between PM_{2.5} mass and urinary MDA mainly during our second sample collection period in mid-pregnancy. This may reflect differences in type of biospecimen. MDA in urine is primarily derived from the kidneys and urinary tract and likely reflects oxidative stress specifically in these tissues, while MDA in serum is derived from various tissues and is a more reliable marker of systemic lipid peroxidation. Differences in exposure assessment such as exposure length may also have contributed to these inconsistencies in findings. Further studies of urinary MDA during pregnancy are needed to identify the periods of pregnancy most affected by short- or medium-term exposure to PM_{2.5} and its speciated PM_{2.5} components.

Our study has several strengths. To our knowledge, this is the first study to assess associations for speciated PM_{2.5} exposures from brake and tire wear in relation to oxidative stress biomarkers during pregnancy. Thus, in addition to the usual PM_{2.5} mass and BC measures employed, we investigated the contributions of brake and tire wear markers and the oxidative potential of the entire particle mixture from different sources. This was possible

due to our novel co-kriging modeling framework that resulted in fine-scale estimates of speciated component from brake and tire wear. Furthermore, having multiple samples available for urinary biomarker measurements allowed us to investigate susceptible windows of exposures across pregnancy. We also obtained all residential addresses during pregnancy, which helps to avoid potential misclassification bias due to mother's change of residence during pregnancy. Additionally adjusting for Alternate Mediterranean Diet scores (aMED) as an indicator for antioxidant intake in a subgroup of 123 women with dietary data did not change our results.

There are also some limitations. First, the monitoring period on which we relied to build our exposure models and the pregnancy periods we applied our modeled estimates to were slightly temporally misaligned. Our model building relied on 2 sampling campaigns (summer 2019 and winter 2020 pre-pandemic), but most of the temporal adjustment relied on low-cost PurpleAir sensor measurements between March 2019 and February 2020 that we used to generate monthly predicted values. These monthly surfaces were then applied backwards to pregnancies from 2016 to 2019, which likely generated some temporal exposure misclassification even though traffic-related PM_{2.5} sources did not change during this period. Second, our limited sample size makes this primarily an exploratory study. Larger prospective studies with multiple sample collection time points are needed and future studies may consider early pregnancy loss. Nevertheless, our study has a relatively large sample size compared to most other studies of this kind. Third, the high socioeconomic status and education of our participants prevents us from drawing conclusions about less-advantaged women and their exposures in LA. This may also have contributed to even lower statistical power in estimating air pollution effects from brake and tire wear. We also did not assess relatively acute exposures shortly before the urine sample collection. In addition, we had fewer urine samples for women during their third trimester compared to the earlier time points ($n = 136, 135, 111$, respectively). This, however, was not due to loss to follow-up and, thus, would not be expected to bias our results, as around 20% of the participants for whom we missed the third sample urine measurement had not yet reached their third trimester by the time we were required to send our samples to the laboratory for biomarker analysis; and for more than 90% of the participants we collected at least 2 samples. Lastly, due to resource constraints, we were unable to assess oxidative protein damage or mtDNA damage in women's serum. Studies evaluating a fuller array of oxidative damage in relation to metal mixtures and oxidative potential from PM_{2.5} are warranted.

Conclusion

Our findings provide novel insights into potential biological mechanism of PM_{2.5} exposure-related health effects among pregnant women, suggesting that even in relatively low particulate level exposure settings, traffic-related air pollution leads to oxidative stress generation during pregnancy, a mechanism previously linked to adverse birth outcomes.

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¹ Study investigators, conference presentations, preprint publication information, thanks.

Supplementary material

Supplementary material is available at *American Journal of Epidemiology* online.

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Conflict of interest

The authors declare no conflicts of interest.

Data availability

Data required to reproduce the findings herein will be shared via the Human Health Exposure Analysis Resource (HHEAR, formerly CHEAR) platform maintained by the National Institute of Environmental Health Sciences (NIEHS) contractors.

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