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Identifying sensitive windows for prenatal particulate air pollution exposure and mitochondrial DNA content in cord blood

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Abstract

Introduction—Changes in mitochondrial DNA (mtDNA) can serve as a marker of cumulative oxidative stress (OS) due to the mitochondria's unique genome and relative lack of repair systems. *In utero* particulate matter ≤2.5μm ($PM_{2.5}$) exposure can enhance oxidative stress. Our objective was to identify sensitive windows to predict mtDNA damage experienced in the prenatal period due to $PM_{2.5}$ exposure using mtDNA content measured in cord blood.

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Material and Methods—Women affiliated with the Mexican social security system were recruited during pregnancy in the Programming Research in Obesity, Growth, Environment and Social Stressors (PROGRESS) study. Mothers with cord blood collected at delivery and complete covariate data were included (n=456). Mothers' prenatal daily exposure to $PM_{2.5}$ was estimated using a satellite-based spatio-temporally resolved prediction model and place of residence during pregnancy. DNA was extracted from umbilical cord leukocytes. Quantitative real-time polymerase chain reaction (qPCR) was used to determine mtDNA content. A distributive lag regression model (DLM) incorporating weekly averages of daily $PM_{2.5}$ predictions was constructed to plot the association between exposure and OS over the length of pregnancy.

Results—In models that included child's sex, mother's age at delivery, prenatal environmental tobacco smoke exposure, birth year, maternal education, and assay batch, we found significant associations between higher $PM_{2.5}$ exposure during late pregnancy (35–40 weeks) and lower mtDNA content in cord blood.

Conclusions—Increased $PM_{2.5}$ during a specific prenatal window in the third trimester was associated with decreased mtDNA content suggesting heightened sensitivity to PM-induced OS during this life stage.

Keywords

particulate matter; mitochondrial DNA; distributive lag models; prenatal exposure

1. Introduction

Prenatal exposure to particulate matter less than 2.5 microns in diameter ($PM_{2.5}$) has been associated with a number of adverse fetal outcomes including reductions in birth weight and preterm birth (Fleisch *et al.* 2015; Hyder *et al.* 2014; Lakshmanan *et al.* 2015; Lamichhane *et al.* 2015; Morello-Frosch *et al.* 2010). The underlying mechanism through which exposure to ambient air pollution leads to adverse fetal outcomes has not been completely elucidated, although oxidative stress (OS) is thought to play a central role (Slama *et al.* 2008). Because mitochondrial DNA (mtDNA) lacks protective histones and has diminished DNA repair capacity compared to nuclear DNA, it is particularly prone to oxidative damage (Shaughnessy *et al.* 2014). In humans, mitochondria contain multiple copies of maternally-inherited double stranded, circular mitochondrial DNA (mtDNA). To maintain optimal physiological functions, mtDNA content (also referred to as mtDNA copy number) is kept within a relatively stable range. Mitochondria are both major intracellular sources of and primary targets of reactive oxygen species (ROS), and are especially susceptible to even small increases in systemic OS (Lee *et al.* 2000; Lee *et al.* 2005; Sinha *et al.* 2013). Mitochondria may respond to increased energy demands by increasing copy number (Lee *et al.* 2000; Lee *et al.* 2005). However, when compensatory mechanisms are overwhelmed, mtDNA content may decrease (Lee *et al.* 2000; Lee *et al.* 2005). Also, mtDNA mutations and their resulting biochemical defects accumulate over time. As such, altered mtDNA content may provide a record of past environmental exposures to pro-oxidant chemicals. Therefore, measurement of mtDNA content in cord blood may also serve to assess a particular vulnerable period in which the fetus is susceptible because of rapid development but also due to immature detoxifying enzyme systems (Wells *et al.* 2009).

Exposure to particulate matter has been associated with changes in mitochondrial DNA content in non-pregnant adults (Hou *et al.* 2010; Hou *et al.* 2013) as well as in cord blood and placenta reflecting *in utero* exposures (Clemente *et al.* 2015; Janssen *et al.* 2012). Measurement of this biomarker in cord blood may provide a source of integrated molecular information of fetal exposures over pregnancy. Studies are starting to emerge that link prenatal pro-oxidant environmental exposures to mtDNA content at birth. a study on prenatal smoking reported decreased placental mtDNA content related to increasing number of cigarettes smoked per day (Bouhours-Nouet *et al.* 2005). Other pro-oxidant exposures such as outdoor air pollutants (e.g., particulate matter,) have also been associated with changes in mtDNA content in the placenta (Clemente *et al.* 2015; Janssen *et al.* 2012). Moreover, there may be periods of time in which individuals are more sensitive to pro-oxidant exposure, such as periods of rapid growth when mitosis is highly active. Exposure to pro-oxidant chemicals during these life stages might induce a cellular response, such as reduced mtDNA copy numbers, that is more prominent than when exposure occurs at other times. For example, development occurs as a cascade of gene expression changes that vary at different life stages. Because the developmental processes and sets of genes expressed differ by developmental stage, potential clues to the underlying biological processes can be inferred simply by understanding what life stages are more sensitive to exposure.

We leveraged daily prenatal $PM_{2.5}$ measures available over pregnancy to more precisely identify sensitive windows in relation to mtDNA content measured in cord blood. We combined these approaches with distributive lag models (DLM) that allow us to statistically model and visualize the exposure timing-dependent pattern of associations. Because there is evidence to suggest sex-specific effects of air pollution exposure during pregnancy (Chiu *et al.* 2016; Hsu *et al.* 2015; Lakshmanan *et al.* 2015) we also examined sex stratified associations.

2. Material and Methods

2.1 Study population

Pregnant women who were receiving prenatal care through the Mexican Social Security System (Instituto Mexicano del Seguro Social –IMSS) between July 2007 and February 2011 were recruited in the Programming Research in Obesity, Growth, Environment and Social Stressors (PROGRESS) study. The IMSS provides healthcare to affiliated private sector employees, the majority low- to middle-income workers and their families. Eligibility criteria were as follows: less than 20 weeks gestation, at least18 years old, planning to stay in Mexico City for the next 3 years, had access to a telephone, had no medical history of heart or kidney disease, did not consume alcohol daily, and did not use any steroid or anti-epilepsy medications. Procedures were approved by institutional review boards at the Harvard School of Public Health, Icahn School of Medicine at Mount Sinai, and the Mexican National Institute of Public Health. Women provided written informed consent.

2.2 Prenatal $PM_{2.5}$ levels

Our group has developed an improved satellite based method to estimate daily $PM_{2.5}$ levels across Mexico City for the years 2004–2014 (Just *et al.* 2015). Ultrasounds were not

routinely performed as standard of care; therefore gestational age was based on last menstrual period (LMP) and by a standardized physical examination to determine gestational age at birth (Capurro *et al.* 1978). If the physical examination assessment of gestational age differed by more than 3 weeks from the gestational age based on LMP, the physical exam was used instead of the gestational age determined by LMP. Daily exposure to $PM_{2.5}$ were then estimated for each cohort participant during pregnancy (i.e., individual-level exposure estimates) using a novel spatio-temporal model that incorporates Moderate Resolution Imaging Spectroradiometer (MODIS) satellite-derived Aerosol Optical Depth (AOD) measurements at a 1× 1 km spatial resolution (Just *et al.* 2015). These remote sensing data are calibrated with municipal ground level monitors of $PM_{2.5}$, land use regression (LUR) variables, and meteorological data to yield estimates of daily residential $PM_{2.5}$ levels for each participant. The model was run using day-specific calibrations of AOD data calibrated against ground $PM_{2.5}$ measurements from 12 monitoring stations covering Mexico City and LUR and meteorological variables (roadway density, temperature, relative humidity, planetary boundary layer and daily precipitation). As in previous studies, mixed effect models with spatial and temporal predictors and day-specific random effects were used to account for temporal variations in the $PM_{2.5}$–AOD relationship. For days without AOD data, the model was fit with a seasonal smooth function of latitude and longitude and time-varying average incorporating local monitoring. Model performance was assessed using monitor-level leave one-out cross-validation; the model performed well with an R^2 of 0.724. Due to day to day variation, daily $PM_{2.5}$ measures were averaged into weekly measurements as in prior work (Chiu *et al.* 2016; Hsu *et al.* 2015). To compare the DLM approach to traditional windows, we also calculated the average PM over clinically defined trimesters (1^{st} trimester: 1–13 weeks, 2^{nd} trimester: 14–27 weeks, 3^{rd} trimester: 28 weeks-delivery).

2.3 Mitochondrial DNA content

Venous umbilical cord blood was obtained at the time of delivery for 531 of the 948 infants born into the study. Most missing samples were due to births occurring late at night or in the very early morning hours or mothers not reporting the start of labor to the study workers. The first 260 whole blood samples were stored in PAXgene™ Blood DNA Tubes (PreAnalytiX GmbH, Hombrechtikon Switzerland) and extracted using a QIAamp DNA Blood Kit (QIAGEN). The DNA was then stored at −80°C prior to analysis. The next 271 samples were extracted by conventional phenol–chloroform method after red cell lysis by a second laboratory. The second laboratory stored the DNA at 4°C. Multiplex quantitative real-time polymerase chain reaction (qPCR) was used to determine mtDNA copy number/content. The copy number was calculated by simultaneously measuring the abundance of two gene targets - one specific to mtDNA 12S ribosomal RNA and one to nuclear DNA (nDNA) - and calculated as the ratio of the abundance of these two genes. The primers for qPCR analysis of mtDNA were: mtF805 5'-CCACGGGAAACAGCAGTGATT-3'and mtR927 5'-CTATTGACTTGGGTTAATCGTGTGA-3'. We used a commercial kit to quantify nDNA (TaqMan RNase P Control Reagents Kit, Applied Biosystems); because this is a commercial kit, the information on primers and probe are protected Quantitative real-time PCR was performed using the Bio-Rad CFX96 Real-time PCR detection system (BioRad, Hercules, CA). The PCR conditions were set up as follows: hot start at 95°C for 15

min, followed by 39 cycles of 95°C for 15 s, 60°C for 1 min, and melting Curve 65°C. A pool of 300 DNA samples was used to construct standard curves for mtDNA/nDNA and data was deemed acceptable if the R^2 of the standard curve was > 0.99 (Zhong *et al.* 2016). To ensure quality control, each plate included 2 negative controls, each sample was run in triplicate and each plate contained five interplate controls. The within-run and between-run coefficients of variation of this assay were 5% and 7%, respectively.

2.4 Covariates

Thirteen variables derived from prenatal questionnaire results were used to classify study participant families into six levels based on the socioeconomic status (SES) index created by the Asociación Mexicana de Agencias de Investigación de Mercados y Opinión Pública (AMAI)(Carrasco 2002). These levels were then collapsed into low, medium, and high socioeconomic status. Only 7 mothers in the original cohort (n=948) reported smoking during pregnancy. Prenatal exposure to environmental tobacco smoke was defined as report of a smoker in the home during the second or third trimester of pregnancy. Maternal education was defined as less than high school, some high school or high school graduate and more than high school at enrollment. Assay batch refers to the plate on which the sample was run and was included in the analysis in order to account for potential batch effects. Inclusion of SES into the model did not significantly alter the results, therefore it was excluded from the final model. The covariates included in the final model were sex, maternal age at delivery, maternal education, prenatal exposure to environmental tobacco smoke, year of delivery and assay batch.

2.5 Statistical Analyses

For these analyses, only children who were born full-term, defined as gestational age greater than or equal to 37 weeks and had complete covariate data were included (n=456). Because DNA was extracted using two different methods, we conducted the following normalization procedure. We calculated the ratio of the geometric mean of the two batches and the inverse was applied to the batch that used the phenol-chloroform method because the QIAamp DNA Kit was shown to provide the more highly reproducible results (Andreu *et al.* 2009). These values were then log-transformed. Variance was not significantly different (Levene test p>0.2) between log-transformed values before and after normalization procedure. Supplemental figure S1 shows the distribution of values before and after the normalization procedure. We fitted distributive lag models (DLMs) to estimate the time-varying association between mtDNA content in cord blood and estimated $PM_{2.5}$ level during a given week in pregnancy as previously described (Chiu *et al.* 2016; Hsu *et al.* 2015). In brief, this method incorporates data from all time points simultaneously and assumes that the association between the outcome and exposure at a given time point, controlling for exposure at all other time points, varies smoothly as a function of time. We fitted the linear distributive lag model $Y_i=\beta_0+\sum_{j=1}^{n}[\alpha_j AP_{ij}]+\beta_1 x_{1i}+\beta_2 x_{2i}+\ldots+\varepsilon_i$, where *APij* is the estimated $PM_{2.5}$ level in week *j* of pregnancy and $x_1 i$, …, *xpi* are the additional covariates for subject I and Yi is the natural log of mtDNA. A maximum lag of 40 weeks was included in the DLM cross-basis starting from the date of estimated conception. For participants with gestational age 37–39

weeks, postnatal exposure was used for missing weeks. Covariates included sex, maternal age at delivery, maternal education, prenatal exposure to environmental tobacco smoke (defined as report of any smoker in the home), year of delivery and assay batch. DLMs that modeled a smooth function using b-splines with 3 degrees of freedom were fit (Gasparrini 2011; Gasparrini *et al.* 2010) chosen due to its parsimony and best AIC value; additional smoothing did not significantly improve the model. A sensitive window was identified when the pointwise 95% confidence bands did not contain zero. DLMs were implemented using the dlnm package version 2.2.6 in R version 3.2.3 (Vienna, Austria) (Gasparrini 2011) and other analyses were performed in SPSS version 23 (Chicago, IL).

3. Results

The majority of participants did not have more than 12 years of schooling (77%) and half were classified as having low socioeconomic status. More than a third of participants reported exposure to a smoker in the home during pregnancy. Other relevant cohort characteristics are shown in Table 1. These baseline characteristics did not differ significantly between those included in these analyses when compared to the remainder of the base cohort, except for maternal age at delivery (see supplemental material, Table S1).

Figure 1 shows the association between a 10 μg/m^3 increase in prenatal $PM_{2.5}$ and cord blood mtDNA content in the sample as a whole, adjusting for maternal age, child's sex, maternal education, prenatal tobacco smoke exposure, year of birth and assay batch. The DLM identified a significant association between increased $PM_{2.5}$ exposure in late pregnancy, specifically 35–40 weeks gestation, and reduced mtDNA content in cord blood.

Figure 2 shows sex-specific associations for $PM_{2.5}$ and mtDNA content in cord blood adjusting for maternal age at delivery, year of birth, maternal education, prenatal exposure to environmental tobacco smoke and assay batch. Distributive lag models run in boys and girls separately, identified a significant association between increased $PM_{2.5}$ and lower cord blood mtDNA content among boys (37–40 weeks gestation) while there were no significant associations among girls. Post-hoc, we fitted a linear regression model including a $PM_{2.5}$ *sex interaction term using $PM_{2.5}$ level averaged over these identified sensitive windows, and we found no significant interaction between $PM_{2.5}$ and sex.

Finally, we assessed the "sensitive window" identified by the DLM by also fitting a linear regression model using $PM_{2.5}$ levels averaged over the time period when the association was significant based on the pointwise 95% CI bounds. We also estimated the association between $PM_{2.5}$ averaged over clinically defined trimesters and over all of pregnancy using this same approach. Figure 3 shows a forest plot comparing the difference in mtDNA content for a 10 μg/m^3 increment in $PM_{2.5}$ averaged over the identified window, the 1st, 2nd and 3rd trimester and averaged over the entire pregnancy. Compared to exposure averaged over trimesters, the DLM window average showed more precise estimates.

4. Discussion

Our findings suggest that prenatal exposure to $PM_{2.5}$ during a specific window in late pregnancy is associated with lower mitochondrial DNA content in cord blood, a marker of

cumulative oxidative stress. In addition, there was a suggestion that $PM_{2.5}$ was more strongly associated with differences in mitochondrial DNA content in boys compared to girls. The observed associations remained significant after adjustment for a number of important potential confounders and covariates. These observations may be particularly relevant for susceptibility to health effects that are mediated by white blood cells, such as infections and immune response to allergens among others. If these findings are replicated in independent studies, future research should determine whether air pollution in this window increases the risk of immunological health effects later in life. Because we only measured mtDNA content in WBCs, the effects of oxidative stress from PM on other health endpoints would be speculative. However, if WBC mtDNA content is a biomarker of cumulative oxidative stress, these data may be useful for establishing the baseline dose of OS and relating the impact of subsequent postnatal PM and ROS-inducing environmental agents. OS plays a major role in many different health endpoints and measures of cumulative OS would have value in epidemiological settings as a means to quantify OS regardless of source, and to determine the contribution of environmental exposures such as PM to cumulative OS.

Previous data on the association between ambient air pollution and mtDNA content has been mixed. In a study of foundry workers in Brescia, Italy, occupational exposure to PM_{10}, PM_1 and coarse particles were associated with higher mitochondrial DNA content in blood (Hou *et al.* 2010). In a multicenter cross-sectional study in Italian cities, personal exposure to benzene was also associated with higher mtDNA content (Carugno *et al.* 2012). More recently, in an occupational study of truck drivers and office workers in Beijing, China, short term (averaged of 2–8 days) personal exposure to elemental carbon (EC) and PM_{10} was associated with lower mtDNA content in all participants (Hou *et al.* 2013). In a panel of elderly people living in Belgium, increased annual $PM_{2.5}$ was associated with decreased mtDNA content in blood (Pieters *et al.* 2015). Only one other study has reported on the association between prenatal PM exposure and mitochondrial DNA content in cord blood (Janssen *et al.* 2012). Janssen *et al* reported that prenatal PM_{10} during the third trimester of in pregnancy was associated with significantly lower mitochondrial DNA content in placenta but no significant associations were reported in mtDNA content in cord blood (Janssen *et al.* 2012). Given these results, the advantages of applying a data-driven method rather than pre-assigned time windows are apparent. Our analyses suggest that the direction of the effect estimates using $PM_{2.5}$ averaged over clinically defined trimesters were generally consistent with the results from DLMs, but the DLMs are more sensitive to the potential significant critical windows. This implies that analyses using exposure metrics arbitrarily defined a priori, such as clinical trimesters, may miss associations if the sensitive window only consists of a portion of a given trimester.

Mitochondrial DNA content can change under different energy demand settings, as mitochondria are the primary source of energy from oxidative phosphorylation. Increases in energy demands can be due in part to high exogenous oxidative stress levels which can overwhelm mitochondrial systems that scavenge endogenous ROS. With prolonged exposure, exogenous ROS can overwhelm the antioxidant capacity of the cell and affect mitochondria quality (Lee *et al.* 2000). Mild oxidative stress due to exogenous factors, like ambient air pollution, may first lead to increased mtDNA synthesis as a compensatory mechanism in order to ensure cell survival (Lee *et al.* 2000; Lee *et al.* 2005). Continued high

exposure and oxidative stress induces mtDNA damage and may result in decreased or limited replication of mtDNA due to increased abundance of defective mitochondria (Lee *et al.* 2005; Meyer *et al.* 2013). Cellular apoptosis can be initiated by damaged mtDNA and feedback mechanisms are designed to limit the replication of damaged mitochondria in order to protect the cell. Unless these defective mitochondria are repaired or removed, production of excess ROS from damaged mitochondrial respiration may continue, resulting in alterations in the bioenergetic and replicative functions of the cell, eventually leading to cell senescence or apoptosis (Lee *et al.* 2000; Lee *et al.* 2005). It is biologically plausible that the results we see are limited to the end of pregnancy due this is the period of fastest growth of the fetus or because there is an accumulation of mtDNA damage over the course of pregnancy via PM exposure. Finally, we cannot rule that the results we see are due to late 3rd trimester exposure being closest in time to the collection of cord blood DNA. It may be that effects are always strongest for most recent exposure. Future work is needed measuring PM and mtDNA content at multiple life stage to determine if the most proximal time of PM exposure is the best predictor of mtDNA content or if earlier life stages predict mtDNA content independent of proximal exposure.

Previous studies have suggested sex-specific differences in response to *in utero* exposure to particulate matter, including differences in neurodevelopmental outcomes (Chiu *et al.* 2016), asthma (Clark *et al.* 2010; Hsu *et al.* 2015), and autism spectrum disorders (Roberts *et al.* 2013). In our analyses, we observed that the effect of prenatal particulate matter may differ by sex, with one potential window of susceptibility detected only in males when compared with females. These results are in line with previous evidence suggesting males may be more susceptible to *in utero* OS than females (Minghetti *et al.* 2013). However, these results should be interpreted with caution given that in our post-hoc analysis we did not find a significant interaction.

Evidence underscores the central role of oxidative stress (Wells *et al.* 2009) and the importance of optimal oxidant balance at the maternal-fetal interface in normal development (Herrera *et al.* 2014). Oxidative stress impacts multiple mitotically stable cellular processes including mtDNA function which have been implicated in programming (Byun *et al.* 2014; Cameron *et al.* 2002; Janssen *et al.* 2014; Shaughnessy *et al.* 2014). Alterations in mtDNA content/mitochondrial dysfunction are associated with fetal outcomes that are important predictors of health later in life like birth weight (Clemente *et al.* 2015) and intrauterine growth restriction (Mando *et al.* 2014). Furthermore, mitochondrial dysfunction is implicated in a variety of diseases and conditions like autism (Chen *et al.* 2015), diabetes and insulin resistance (Wong *et al.* 2009; Zheng *et al.* 2015), glucose metabolism (Weng *et al.* 2009), cognitive function (Lee *et al.* 2010; Mengel-From *et al.* 2014), and chronic kidney disease (Tin *et al.* 2016).

This study has several strengths. First, we were able to use a novel spatio-temporal satellite method of high spatial and temporal resolution to assign exposure during pregnancy. We leveraged this time-varying exposure data to highlight new data-driven statistical methods that can better delineate the role of exposure timing in air pollution health effects. We were also able to adjust for important confounders. We also acknowledge some limitations. We did not consider air pollution exposures in microenvironments that might lead to personal

exposure levels that differed from our ambient exposure estimated at the home. We were not able to adjust for other important traffic-related pollutants like NO_2. Another limitation is that we were not able to adjust for blood cell count, particularly platelet count, which is thought to influence variation in mtDNA content by increasing mtDNA without increasing nDNA(Banas *et al.* 2004). The mtDNA content biomarker would capture effects in both WBCs and platelets and our results are therefore a reflection of the relative contribution of each cell type to the overall mtDNA content. However, Janssen et al (Janssen *et al.* 2012)reported that none of the blood cell count measures included in their models (platelets, neutrophils, white blood cells or white blood cell ratio were not significant predictors of mtDNA content in cord blood. Also, we cannot rule out potential residual confounding due to unmeasured host and environmental factors that may influence mitochondrial DNA content in cord blood. However, the specificity of our findings to a limited period within pregnancy suggests that such an unmeasured confounder would likely need to vary in time along with $PM_{2.5}$ and would not be explained by more time-invariant characteristics like socio-demographics. For children born before 40 weeks, the period of exposure is not limited to *in utero* but includes early postnatal exposure. Finally, the cohort consisted Mexican women belonging mostly to low-income families, in a megacity with substantial $PM_{2.5}$ exposures that may limit the generalizability of our findings.

In our study, increased $PM_{2.5}$ at the end of the third trimester was associated with decreased mtDNA content, suggesting heightened sensitivity to PM during this particular time period. Refined determination of time windows in which PM has the greatest magnitude of effect can enhance insight into underlying mechanisms and may better inform future interventions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

DLM	Distributive lag models
mtDNA	Mitochondrial DNA
$PM_{2.5}$	Particulate matter <2.5 microns in diameter
qPCR	Quantitative real time polymerase chain reaction
ROS	Reactive oxygen species

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Highlights

- Examined sensitive windows of prenatal $PM_{2.5}$ exposure on mtDNA in cord blood.
- Sensitive window identified at gestational weeks 35–40
- Higher $PM_{2.5}$ during sensitive window associated with lower mtDNA content.
- Findings suggest sex-specific associations.

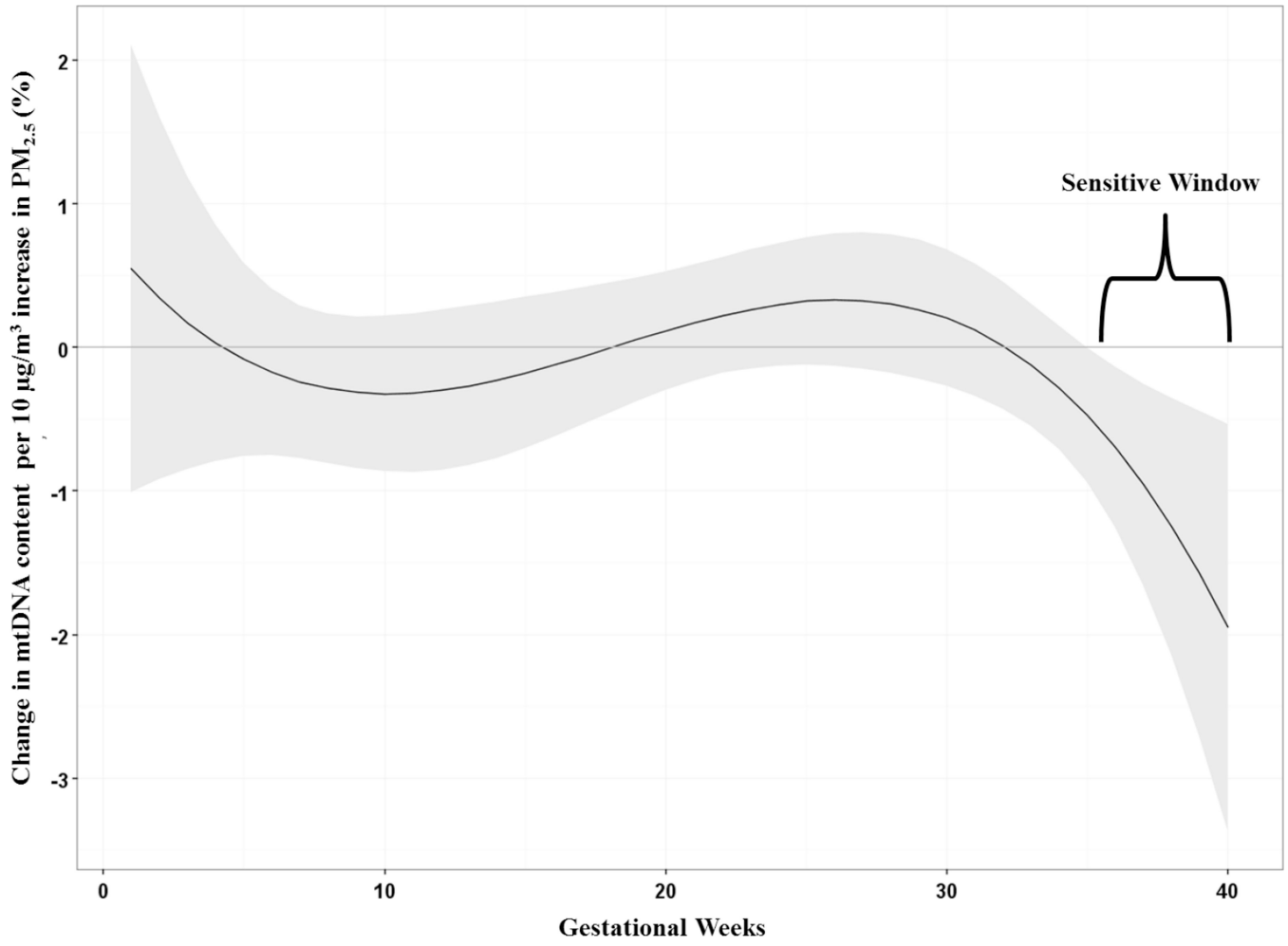

Figure 1.

Associations between weekly prenatal $PM_{2.5}$ and mtDNA content in cord blood in the entire sample (n=456). Adjusted for sex, maternal age at delivery, year of birth, maternal education, prenatal exposure to environmental tobacco smoke and batch. The y-axis represents the change in mtDNA content associated with a 10 μg/m^3 increase in $PM_{2.5}$; the x-axis is gestational age in weeks. Solid lines show the predicted change in mtDNA content. Gray areas indicate 95% CIs. A sensitive window is identified for the weeks where the estimated pointwise 95% CI (shaded area) does not include zero.

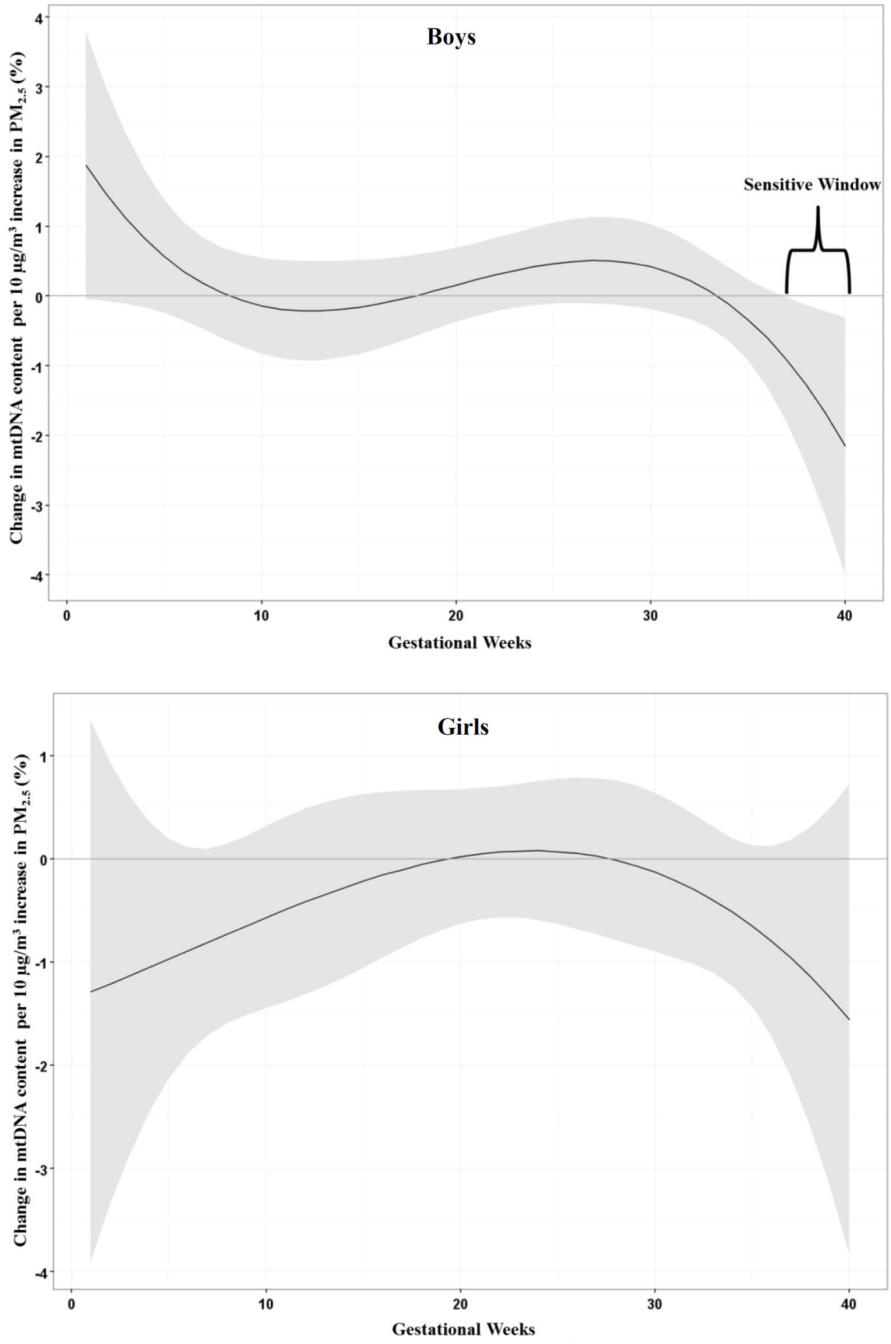

Figure 2.

Sex-stratified associations between weekly prenatal $PM_{2.5}$ and mtDNA content in cord blood. Adjusted for maternal age at delivery, year of birth, maternal education, prenatal exposure to environmental tobacco smoke and assay batch. The y-axis represents the change in mtDNA content associated with a 10 μg/m^3 increase in $PM_{2.5}$; the x-axis is gestational age in weeks. Solid lines show the predicted change in mtDNA content. Gray areas indicate 95% CIs. A sensitive window is identified for the weeks where the estimated pointwise 95% CI (shaded area) does not include zero

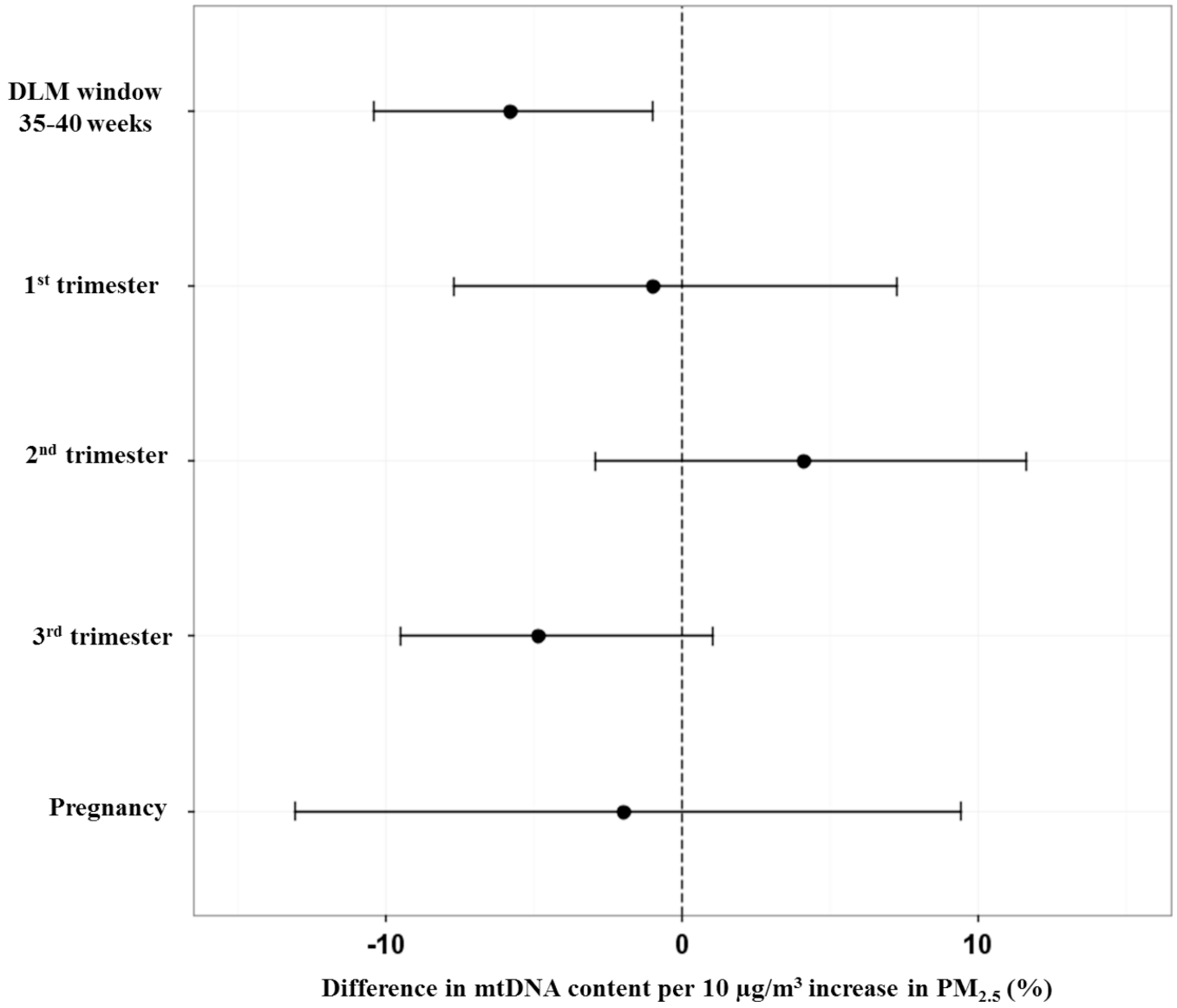

Figure 3.

Comparison of linear model estimates for percent difference in mtDNA content per 10 $\mu g/m^3$ higher $PM_{2.5}$ concentration averaged over the DLM defined window, the clinically defined trimesters and the entire pregnancy. All models adjusted for child's sex, maternal age at delivery, maternal education, prenatal exposure to environmental tobacco smoke, year of birth and assay batch.

Table 1

PROGRESS cohort characteristics

Characteristic	N=456
Child's sex, n (% male)	252 (55)
Prenatal ETS exposure, n (%)	160 (35)
Maternal education at enrollment	
Less than high school, n (%)	181 (40)
Some high school or high school graduate, n (%)	170 (37)
More than high school, n (%)	105 (23)
Socioeconomic status	
Low, n (%)	235 (52)
Medium, n (%)	171 (37)
High, n (%)	50 (11)
Maternal age at enrollment years, median (25^{th}–75^{th})	28.0 (24.4–35.5)
Average prenatal $PM_{2.5}$ $\mu g/m^3$, median (25^{th}–75^{th})	23.1 (20.8–24.5)
Normalized relative cord mtDNA content, geometric mean (25^{th}–75^{th})	1.13 (1.00–1.32)

[a] Differences in categorical variables tested using Pearson Chi-Square, differences in continuous variables tested using Mann Whitney U test