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The cross-sectional and longitudinal association between air pollution and salivary cortisol: Evidence from the Multi-Ethnic Study of Atherosclerosis

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Abstract

Background—Cortisol, a stress hormone released by the activation of the hypothalamic-pituitary-adrenal (HPA) axis, is critical to the body's adaptive response to physiological and psychological stress. Cortisol has also been implicated in the health effects of air pollution through the activation of the sympathetic nervous system. This study evaluates the cross-sectional and longitudinal association between several air pollutants and salivary cortisol.

Methods—We used data from the Multi-Ethnic Study of Atherosclerosis (MESA), a cohort of 45 – 85 years old participants from six US cities. Salivary cortisol was evaluated at two time points between 2004 – 2006 and then again from 2010 – 2012. Cortisol samples were taken several times per day on two or three consecutive days. Particulate matter less than 2.5 μm in diameter ($PM_{2.5}$), nitrogen dioxide (NO_2) and nitrogen oxides (NO_x) in the year prior to cortisol sampling were examined. We used piecewise linear mixed models that were adjusted for demographics,

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socioeconomic status and cardiovascular risk factors to examine both cross-sectional and longitudinal associations. Longitudinal models evaluated change in cortisol over time.

Results—The pooled cross-sectional results revealed largely null results with the exception of a 9.7% higher wake-up cortisol associated with a 10 ppb higher NO_2 (95% CI: −0.2%, 20.5%). Among all participants, the features of the cortisol curve became flatter over 5 years. The wake-to-bed slope showed a more pronounced flattening over time (0.014, 95% CI: 0.0, 0.03) with a 10 ppb higher NO_2 level. Other air pollutants were not associated with change in cortisol over time.

Conclusions—Our results suggest only a moderate association between traffic related air pollution and cortisol. Very few epidemiologic studies have examined the long-term impact of air pollution on the stress response systems, thus warranting further exploration of these findings.

Keywords

cortisol; $PM_{2.5}$; NO_2; traffic related air pollution; sympathetic nervous system; hypothalamic-pituitary-adrenal axis

1. Introduction

One hypothesized mechanism by which air pollution results in acute cardiovascular health effects is by creating an imbalance of the autonomic nervous system (ANS), with activation of the sympathetic nervous system (SNS) and the withdrawal of the inhibitory effects of the parasympathetic nervous system (PSNS) (Brook et al. 2010). The ANS is also central to the body's response to psychosocial stress. Here the activity of the hypothalamic-pituitary-adrenal (HPA) axis, the other central component of the stress response system, plays a critical role in response to both acute and chronic stress. These two systems, the ANS and the HPA, are tightly interconnected through reciprocal innervation and through hormone production as exemplified by the existing feedback mechanisms between the two systems e.g. an external stressor increases norepinephrine (NE) production from the SNS which then stimulates secretion of corticotrophin-releasing hormone (CRH) from the hypothalamus, which ultimately triggers cortisol secretion (Rotenberg and McGrath 2016; Sapolsky et al. 2000). This strong connection between the HPA and the ANS provides rationale for examining the association between air pollution and cortisol, a stress hormone released by the HPA axis.

Air pollution induced inflammation either via ANS imbalance or via lung inflammation that spills over into systemic inflammation has been hypothesized and documented (Brook et al. 2010). In response to psychosocial stress, cortisol is also known to suppress immune and inflammatory response and is important in regulating cardiovascular and metabolic systems (Black and Garbutt 2002; Padro and Sanders 2014; Uchoa et al. 2014). Recent work suggests that inhaled pollutants interact with the lung to directly activate the HPA axis which then mediates lung inflammation (Snow et al. 2018). Thus the role of cortisol in immune and inflammatory response provides further justification for its study in relation to air pollution.

Several animal studies have shown changes in cortisol and related hormones following short-term exposure to fine particulate matter ($PM_{2.5}$) (Balasubramanian et al. 2013; Sirivelu et al. 2006; Thomson et al. 2013), implying activation of the HPA. In addition to these acute

effects, there is reason to believe that long-term exposure to air pollution could also result in chronic dysregulation of the HPA axis. As has been shown with exposure to chronic stress, repeat exposure to stressors or sustained periods of stress can result in dysregulation of the HPA axis as well as altered immune and inflammatory responses (Black and Garbutt 2002; McEwen and Seeman 1999). Exposure to air pollution, even at low doses, occurs on a daily basis; thus the accumulation of air pollution's negative health effects over time may have similar effects to those of chronic stress.

The objective of this study is to evaluate the association between exposure to long-term air pollutants and the stress hormone cortisol. This research has implications for several evolving lines of research. Identifying biomarkers that are activated in response to both stress and air pollution can inform the emerging research on joint effects of air pollution and psychosocial stress on health. In addition, this research could provide support for the direct link between air pollution and HPA activation as has been shown in animal studies (Kodavanti 2016; Snow et al. 2018). Given the important role of the HPA in several chronic health conditions, a better understanding of the link between air pollution and the HPA is a critical area for future research.

2. Material and Methods

2.1 Study Population

The study population was drawn from the Multi-Ethnic Study of Atherosclerosis (MESA) (Bild et al. 2002). MESA is a prospective cohort study in six U.S. cities, which recruited adults aged 45–84 from four racial/ethnic groups who were free of clinical cardiovascular disease (CVD) at baseline (2000–2002). Six clinical exams have been conducted thus far in the MESA cohort. The MESA Stress study is an ancillary study to MESA that occurred during 2004–2006 (Stress I), aligning with MESA exams 3 and 4, and again during 2010–2012 (Stress II), aligning with MESA exam 5. For Stress I, MESA participants from the New York, NY and Los Angeles, CA study sites were recruited. Stress II included a subset of participants from Stress I (about half of the participants were the same), additional MESA participants at the two Stress I study sites, and MESA participants from the Baltimore, MD study site. Institutional review board approval was granted at each study site and written informed consent was obtained from participants.

2.2 Air Pollution Exposure

The MESA Air Pollution Study (MESA Air), another ancillary study to MESA, has investigated the impact of air pollution exposures on subclinical and clinical CVD in the MESA cohort (Kaufman et al. 2012). Long-term estimates of air pollution exposure for individuals in the MESA Air cohort were generated using spatio-temporal air pollution modeling in each study area (Keller et al. 2014). These models utilize regulatory monitoring network data collected by the U.S. Environmental Protection Agency Air Quality Systems, cohort-specific air monitoring, and a suite of geographic covariates. Exposures to fine particulate matter ($PM_{2.5}$), oxides of nitrogen (NO_x) and nitrogen dioxide (NO_2) are predicted at participants' homes. As secondary metrics of interest, data on residential infiltration and time-location patterns were used to predict individual-level exposure to

ambient $PM_{2.5}$ (Allen et al. 2012; Cohen et al. 2009) and light absorbing carbon was used to represent black carbon (BC). Unlike the other long-term air pollutants, the BC measure did not vary with time. Long-term exposure is represented by the annual average of exposure in the year prior to each Stress Study visit. Participants that moved within the MESA study area in the year prior to their Stress Study visit have data incorporated for their new address. Participants that moved outside the study area, provided addresses that we were unable to geocode or provided PO Boxes were not included in our study.

2.3 Salivary Cortisol

Salivary cortisol was collected for MESA Stress study participants at Stress I and Stress II. To account for the strong diurnal pattern, salivary cortisol was collected at six time points throughout the day (upon awakening, 30 minutes after wake-up, around 1000 h, around 1200 h, around 1800 h and before bed time) for three consecutive days during Stress I and eight times throughout the day (the same times as Stress I with the addition of 1 hour after breakfast and around 1600 h) for two consecutive days during Stress II. Saliva samples were collected using Salivette® collection tubes which include a cotton swab to be placed inside the mouth. Participants were asked to store saliva samples in their refrigerators before returning them to the clinic. For most participants, time between collection and return ranged from a few days up to a week. Salivary cortisol has been shown to be stable at room temperature for up to several days, thus refrigerator storage likely did not degrade the quality of the sample (Clements and Parker 1998). Upon receipt, saliva samples were stored at −20 °C until analysis. Samples were thawed and centrifuged at 3000 rpm for three minutes to obtain clear saliva with low viscosity. Cortisol levels were determined by employing a commercially available chemi-luminescence assay (CLIA) with a high sensitivity of 0.16 ng/mL (IBL-Hamburg, Germany); assays were evaluated at a central laboratory in Dresden Germany. Intra-and inter-assay coefficients of variation were below 8%.

In order to assess compliance with study protocol, for participants in Stress Study I we used time tracking devices (known as track caps) that automatically registered the time at which the Salivette cotton swab was removed. Participants were informed of this device. It has been shown that use of these devices improves compliance with study protocol (Kudielka et al. 2003). We did not use these devices during Stress Study II due to costs. Device times were compared against self-reported wake-up and sample collection times to better assess compliance to the protocol.

2.4 Covariates

Age, race/ethnicity (non-Hispanic white, non-Hispanic black, or Hispanic), gender, study site, work outside of the home (including volunteer work), income-wealth index (Hajat et al. 2010) and education (less than high school, some college, at least a college degree) were included as covariates. A neighborhood deprivation index (NDI) was calculated based on census tract level data after conducting a principal components analysis and has been used in other MESA analyses (Christine et al. 2015). The following CVD risk factors were also included: body mass index (BMI in kg/m^2), smoking status (current, former, or never smoker), second-hand smoke exposure (SHS, yes/no) and current alcohol use (yes/no).

Wake-up time on the day of saliva sampling (centered at 700 h) was also included in all models.

Psychosocial stress variables including depression, anxiety, chronic burden, and emotional support were also included as covariates. Depression was measured at each exam by the 20-item Center for Epidemiologic Studies Depression scale and dichotomized so that values greater than or equal to 16 were considered depressed (Radloff 1977). Trait anxiety was measured with the continuous Spielberger anxiety scale, at MESA exam three (Spielberger 1980). Chronic burden was also measured at MESA exam three using a five-item scale assessing difficulties in five separate domains of life (Bromberger and Matthews 1996). Missing values for anxiety or chronic burden at exam three were imputed using that participant's data from MESA exam one. Emotional support was calculated at each exam using the six-item ENRICHD social support scale and one item about marital status (Mitchell et al. 2003).

2.5 Statistical Analyses

Salivary cortisol sampling days were excluded from analysis if there was no time since wake-up recorded for the entire day or if there were no cortisol values for the entire day. Individual salivary cortisol samples were excluded if they were missing a cortisol value, the time the saliva sample was taken, or if they had unreliable cortisol values (>100 or 0 nmol/L). Participants were excluded from analyses if they were on hormone replacement therapy (defined as use of estrogen and/or progestin), used inhaled or oral steroids or were missing air pollution predictions, usually as a result of missing or highly imprecise geocoded residential address data.

Salivary cortisol curves were modeled using piecewise linear mixed models, which capture the non-linear pattern of cortisol and simultaneously account for within- and between-person variation in cortisol levels. We placed knots at 30 and 120 minutes after wake-up which allows for the estimation of three pieces (or slopes/features) of the cortisol curve: cortisol awakening response (CAR) which is the slope from wake-up to 30 minutes after wake-up; early decline which is the slope from 30 minutes to 2 hours after wake-up; and late decline which is the slope from 2 hours after wake-up to bed time (Hajat et al. 2010). We also evaluated two summary measures of the diurnal curve: wake-to-bed slope and area-under-the-curve (AUC). Lastly, we evaluated wake-up and bedtime cortisol levels, resulting in a total of seven outcome measures for salivary cortisol. Salivary cortisol in nmol/L was log-transformed (ln(x)) due to excessive skewness and cross-sectional parameter estimates were presented as percent differences.

We evaluated both cross-sectional and longitudinal associations between cortisol and air pollution. In the cross-sectional analysis data from Stress I and Stress II were pooled to increase power. Interaction terms between air pollutants and the three slopes were included to estimate associations of air pollution with each feature of the curve. All covariates were also interacted with slopes to adjust for confounding. Random intercepts and slopes for the first (i.e. cortisol awakening response) and third (i.e. late decline) slopes were used to adjust for both within and between-person clustering. Including random effects for all threes slopes lead to model non-convergence; however, results were similar regardless of which two

slopes were specified as random. Furthermore, a random effect for stress study indicator (Stress I or II) was included to account for within study correlation. Lastly, in order to adjust for day-level variability we indicated a fixed effect for day of week the sample was taken (Sanchez et al. 2012).

The piecewise linear mixed model has been adapted to evaluate change in cortisol over time for those participants enrolled in both Stress I and Stress II (Hajat et al. 2015b). These models evaluate 5-year change in cortisol in relation to annual average air pollution exposures prior to Stress I for each participant. Using exposures prior to Stress I better captures our goal of evaluating the association between long-term air pollution and cortisol. The 5-year change in cortisol reflects the approximate time between the two Stress Studies. Income-wealth index, work outside the home, BMI, smoking status, second-hand smoke exposure, and alcohol use were allowed to vary with time. We used age at the time of Stress Study I since time between visits was included as a covariate. Similar to the cross-sectional models, air pollution exposures and covariates were interacted with slopes. Time between visits was also interacted with slopes in order to evaluate the average change in each piece of the cortisol curve over time. Similarly time between visits was interacted with air pollution exposure and covariates significant at the 0.2 p-value level. A three-way interaction between exposure, slopes and time between visits was used to assess the departure from this average change. Lastly three-way interactions between covariates, slopes and time were included in the longitudinal model only if significant at the 0.2 p-value level. Random intercepts and slopes were similar to the cross-sectional model, with the inclusion of a random effect for time and a random effect for the interaction between time and the first and third slope. The random effect for stress study indicator was not needed in this model.

For all analyses, we used a staged modeling approach starting with a minimally adjusted model and adding more covariates to subsequent models. Model 1 included adjustment for age, sex, race/ethnicity, study site and wake-up time on the day of sampling. Model 2 included income-wealth index, education, employment, and NDI in addition to covariates from model 1 and is our *a priori* primary model of interest. Model 3 included BMI, smoking, second hand smoke and alcohol consumption, as well as depression, anxiety, chronic burden and emotional support in addition to covariates in model 2. Concerns about over-adjustment drove the decision not to include Model 3 covariates as part of our *a priori* primary model. All covariates were mean-centered to allow for population average interpretations. Parameter estimates from piecewise regression models can be interpreted as corresponding to a 5 $\mu g/m^3$ higher $PM_{2.5}$, 40 ppb higher NO_x, 10 ppb higher NO_2 and 0.5 $10^{-5}m^{-1}$ higher black carbon level. All analyses were implemented using SAS 9.4 (SAS Institute Inc., Cary, NC).

We also conducted sensitivity analysis using the year 2000 air pollution estimates for the longitudinal cortisol analyses to alternatively assess long-term air pollution levels and to reduce potential bias from secular trends in air pollution.

3. Results

After exclusions for cortisol, air pollution and missing covariates, there were 867 Stress Study I participants and 926 from Stress Study II (n = 1793 in pooled sample) and 491 in

both (Table 1 and Supplemental Table S1). The study population was about half female, with an average age of 65 years at Stress I. The pooled sample was about 47% Latinx and about 30% black. The sample for Stress II included a lower proportion of Latinx participants due to the inclusion of the Baltimore study site. Slightly less than half of the pooled sample participants had less than a high school degree (44%) and worked outside the home (48%). Mean BMI was 29 kg/m^2 for both the pooled and longitudinal samples, while the other CVD risk factors, smoking, SHS exposure and alcohol use, declined slightly over time. The psychosocial stressors were relatively consistent when comparing the pooled and longitudinal samples. As expected, we observe a decreasing secular trend in all pollutant exposures over time, with the exception of BC which was measured at only one point in time (Table 2).

Exclusions based on cortisol sampling are described in the supplementary material (Table S1). In the pooled sample, average cortisol levels were about 10 nmol/L, wake-up cortisol was 15 nmol/L and wake-to-bed slope declined over the course of the day (means are negative). In the longitudinal analysis, average and wake-up cortisol as well as AUC increased and the slope became flatter over time (Table 2).

As shown in Table 3, the pooled cross-sectional results from Model 2 revealed largely null results with the exception of a 9.67% higher wake-up cortisol associated with a 10 ppb higher NO_2 (95% CI: −0.16%, 20.47%). Adjustment for additional covariates slightly improved the precision of the estimate (model 3: 9.89% (95% CI: 0.09%, 20.65% shown in Supplemental Table S2)).

In the longitudinal analysis, as expected, the features of the cortisol curve became flatter and the total AUC increased over 5 years (Table 4). Wake-to-bed slope showed a more pronounced flattening over time (0.014, 95% CI: 0.00, 0.028) with a 10 ppb higher NO_2 level. Adjustment for additional covariates slightly attenuated the estimate (Model 3 in Supplemental Table S3, 0.012, 95% CI: −0.002, 0.026). Other air pollutants, however, were not associated with the flattening of the cortisol curve over time; that is, other pollutant findings were null.

Sensitivity analyses using year 2000 air pollution predictions did not substantially impact reported results. The finding of a more pronounced flattening of the wake-to-bed slope in the longitudinal analysis of NO_2 and cortisol remained significant even when year 2000 NO_2 values were used (0.015, 95% CI: 0.001, 0.029) (see Supplemental Table S4).

4. Discussion

In our epidemiologic study of the association between ambient air pollutants and cortisol we observed largely null results in the cross-sectional findings with the exception of higher wake-up cortisol associated with higher NO_2. There was also evidence to suggest that higher NO_2 was associated with a flatter wake-to-bed slope in the longitudinal analysis.

This flattening of the diurnal cortisol slope is similar to results from a recent epidemiologic study among adolescents, where a cross-sectional association between higher long-term NO_2 exposures and flattening of the slope of the cortisol curve were observed; however, no

association was seen with other features of the cortisol curve and these investigators did not report longitudinal findings (Wing et al. 2018). The flattening of the cortisol curve has also been demonstrated with exposures to other chronic stressors and is generally considered evidence of an unhealthy or dysregulated HPA axis (Cohen et al. 2006; Hajat et al. 2010; Karlamangla et al. 2013; Miller et al. 2007). Similar to our study Wing et al. did not find associations with other air pollutants i.e. $PM_{2.5}$ and ozone (2018). NO_2, a marker of traffic related air pollution, has been consistently associated with a variety of health effects (Chan et al. 2015; Faustini et al. 2014; Hajat et al. 2015a; Jacquemin et al. 2015; Su et al. 2015). In MESA Air it is likely that our NO_2 models are better able to assess intra-area differences in TRAP given the study's focus on evaluating roadway gradients (Cohen et al. 2009).

The finding of higher wake-up cortisol associated with higher NO_2 observed in our cross-sectional analysis has not been previously demonstrated with respect to air pollution. Cortisol studies have, however, found that *lower* wake-up cortisol is associated with deleterious exposures, such as low individual and neighborhood SES, race/ethnic minority status and higher levels of inflammatory biomarkers (DeSantis 2012; Do et al. 2011; Hajat et al. 2010; Karlamangla et al. 2013). Lower wake-up cortisol levels tend to contribute to flatter overall slopes over the course of the day. Thus our finding of higher wake-up cortisol is unexpected.

We cannot be certain why we see these unexpected results; however, one possibility is the presence of measurement error, specifically non-differential misclassification of the outcome, i.e. we believe the mismeasurement of cortisol is unrelated to levels of NO_2. Given the extensive nature of our saliva collection protocol (six samples for three days for Stress I and eight samples for two days for Stress II), some participants likely collected samples at times earlier or later than directed. This is especially important for wake-up cortisol which were supposed to be taken immediately upon awakening. Previous research from the MESA Stress Study has shown that wake-up cortisol levels and the CAR are most affected by non-adherence to the protocol (Golden et al. 2013). Providing a sample as few as 10 minutes after wake-up will result in a higher wake-up cortisol value than expected. An evaluation of the times at which wake-up cortisol samples were taken showed that overall the first sample was taken more than 5 minutes after wake-up on 25% of sampling days. Older participants had worse compliance (about 50% of days were later than 5 minutes after wake-up).

Non-differential misclassification of the outcome only results in bias toward the null under certain specific conditions (Jurek et al. 2008; Jurek et al. 2005). Given the other biases at play (e.g. unmeasured confounding, measurement error of the covariates) it is difficult to predict the direction of the bias in our study. Thus, the observed association between higher wake-up cortisol levels and NO_2 may be a result of non-differential misclassification of the outcome. Outcome misclassification in the longitudinal findings is potentially less of a problem since participants had experience with the cortisol protocol in Stress Study I. Previous research has shown that both cortisol patterns and the distribution of air pollution differ for blacks and Hispanics compared to whites and for low vs high SES sub-groups (Hajat et al. 2010; Hajat et al. 2013; Jones et al. 2014). Similarly, research has consistently shown that those with depression, anxiety and PTSD have dysregulated cortisol profiles relative to those without these mental health conditions (Gold 2015; Juruena et al. 2018;

Speer et al. 2019; Zorn et al. 2017). Additional effect modification analyses could help understand these effects, however, given our relatively small sample size and the complexity of our models such analyses in the MESA Stress Study resulted in highly imprecise estimates and in some cases models that did not converge. Therefore, future studies with more robust data may be better poised to evaluate differences in the air pollution – cortisol association by race, SES and pre-existing mental health condition.

Regardless of these potential biases, our study contributes substantially to the body of epidemiologic research on air pollution and the HPA axis. Other epidemiologic studies have compared cortisol and a precursor of cortisol, adrenocorticotropic hormone (ACTH), among indoor and outdoor police workers in Italy and have found outdoor workers to have higher plasma ACTH and cortisol levels (Rosati et al. 2011; Tomei et al. 2003a; Tomei et al. 2003b). Although it is difficult to put a single plasma cortisol sample into the context of a daily cortisol pattern (i.e. it is unclear where one plasma cortisol level falls with respect to the overall diurnal curve), the authors attribute these higher hormone levels to the interaction between chemical, physical and psychosocial stress involved with outdoor police work.

Most other existing research has evaluated acute or short-term impacts of air pollution on stress hormones. A randomized control trial found levels of cortisol, corticotropin-releasing hormone (CRH) (another precursor of cortisol) and ACTH to be higher among participants who received sham versus real air purification systems after 9 days of using the filtration system (Li et al. 2017). A controlled exposure study similarly found significant declines in blood cortisol one and 21 hours after exposure to course PM (an unhypothesized finding) and observed increases in urinary cortisol 21 hours after exposure (Liu et al. 2017). Several animal studies have also found acute effects. One study observed higher CRH levels after one day of exposure to $PM_{2.5}$ (Balasubramanian et al. 2013), while others found CO_2, $PM_{2.5}$ and ozone exposure (administered separately) increased plasma corticosterone (Martrette et al. 2017; Martrette et al. 2011; Sirivelu et al. 2006; Thomson et al. 2013). We did not evaluate short term air pollution concentrations in our study since data from MESA Air were not optimized to do so. Short term concentrations from MESA would reflect only temporal variability between participants and cannot capture fine grain spatial differences.

This study should be viewed in light of its limitations. Our exposure assessment only captures air pollution levels outside one's residential address which omits ambient exposures encountered at work or other locations. These alternative locations may be particularly important for stress hormones such as cortisol. Furthermore, chronic exposure to air pollution over the life course may be most relevant to capturing the full impacts of air pollution's health effects (Brook et al. 2010); however, we did not have life course measures of air pollution. Our air pollution metrics measure annual averages, which we believe are proxies for longer-term exposures. In addition, residual and unmeasured confounding may be biasing our results. Specifically noise was not measured in our study and is likely a confounder of the air pollution – cortisol association. Furthermore, residual confounding by psychosocial stress cannot be overlooked regardless of the stress variables we were able to capture in our study. We also suffer from multiple comparisons; the association between NO_2 and flatter wake-to-bed slope may be due to chance alone. Lastly, our study is likely

not generalizable to a broader population because of its demographic and geographic composition.

Our study has several strengths, namely a large well-characterized longitudinal cohort with excellent exposure and outcome ascertainment. Furthermore, MESA is one of the few studies to have longitudinal measures of cortisol which provides a more robust measurement of physiological change over time.

Our study contributes to the emerging research of air pollution and its impacts on the HPA axis. This line of epidemiologic research while still in its infancy could benefit from studies conducted in both older and younger populations with longitudinal measures of cortisol collected in a rigorous manner, and examination of additional air pollutants. In addition, this work further extends research on joint effects of chemical and non-chemical stressors by pointing to specific mechanisms that may be at play.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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List of abbreviations

HPA	hypothalamic-pituitary-adrenal axis
ANS	autonomic nervous system
SNS	sympathetic nervous system
PSNS	parasympathetic nervous system
MESA	Multi-Ethnic Study of Atherosclerosis
$PM_{2.5}$	particulate matter less than 2.5 μm in diameter
NO_2	nitrogen dioxide
NO_x	nitrogen oxides
BC	black carbon

LDL	low-density lipoproteins
HDL	high-density lipoproteins
NDI	neighborhood deprivation index
SHS	second-hand smoke exposure
BMI	body mass index
AUC	area-under-the-curve
CAR	cortisol awakening response

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Highlights

- NO_2 was associated with flattening of wake-to-bed slope in longitudinal analysis
- NO_2 was associated with higher wake-up cortisol in cross-sectional analysis
- No other pollutants were associated with other features of cortisol curve

Table 1.

Demographic, cardiovascular and psychosocial factors for MESA Stress Study participants[a]

	Stress Study I	Stress Study II	Sample in pooled cross-section	Sample in longitudinal analysis	
				At Stress I	At Stress II
N	867	926	1793	491	491
Demographics					
Female *(n [%])*	430 (49.6)	489 (52.8)	919 (51.3)	241 (49.1)	241 (49.1)
Age (yrs, *mean [SD]*)	65.5 (9.8)	69.6 (9.0)	67.6 (9.6)	63.8 (9.2)	69.4 (9.2)
Race/ethnicity *(n [%])*[b]					
Black	239 (27.6)	305 (32.8)	544 (30.3)	133 (27.1)	133 (27.1)
Hispanic	467 (53.9)	381 (41.4)	848 (47.3)	273 (55.6)	273 (55.6)
White	161 (18.5)	240 (25.9)	401 (22.4)	85 (17.3)	85 (17.3)
Study Site *(n [%])*					
New York	457 (52.7)	415 (44.8)	872 (48.6)	269 (54.8)	269 (54.8)
Los Angeles	410 (47.3)	292 (31.5)	702 (39.2)	222 (45.2)	222 (45.2)
Baltimore	--	219 (23.7)	219 (12.2)	--	--
Education *(n [%])*[c]					
≤ High school degree	418 (48.2)	377 (40.8)	795 (44.4)	228 (46.4)	228 (46.4)
Some college	246 (28.4)	271 (29.3)	517 (28.9)	142 (28.9)	142 (28.9)
≥ College degree	203 (23.4)	276 (29.9)	479 (26.7)	121 (24.6)	121 (24.6)
Working outside the home *(n [%])*	429 (49.5)	425 (46.0)	854 (47.7)	264 (53.8)	229 (44.1)
Not working outside the home *(n [%])*	438 (50.5)	500 (54.0)	938 (52.3)	227 (46.2)	271 (44.8)
Income-wealth index *(mean [SD])*	3.8 (2.3)	2.2 (1.2)	3.0 (2.0)	4.0 (2.2)	2.2 (1.2)
Neighborhood deprivation index (mean [SD])	0.89 (6.93)	0.34 (6.65)	0.61 (6.79)	1.01 (6.83)	1.01 (6.83)
CVD risk factors					
BMI, *(mean [SD])*	29.0 (5.5)	29.4 (5.5)	29.2 (5.5)	29.0 (5.2)	29.2 (5.6)
Smoking Status *(n [%])*[d]					
Current smokers	73 (8.4)	56 (6.1)	129 (7.2)	40 (8.2)	30 (5.8)
Former smokers	398 (45.9)	448 (48.4)	846 (47.2)	211 (43.0)	255 (49.1)
Never smokers	391 (45.4)	418 (45.3)	809 (45.4)	238 (48.7)	224 (45.8)
Second-hand smoke exposure *(n [%>])*	273 (31.9)	186 (20.3)	459 (25.9)	152 (31.3)	92 (18.9)
No second-hand smoke exposure *(n [%>])*	586 (68.2)	730 (79.7)	1316 (74.1)	333 (68.7)	396 (81.1)
Alcohol use *(n [%])*	380 (43.9)	354 (38.4)	734 (41.1)	230 (46.8)	186 (36.0)
No alcohol use *(n [%])*	486 (56.1)	568 (61.1)	1054 (58.9)	261 (53.2)	315 (64.4)
Psychosocial stress					
Depressed *(n [%])*[e]	160 (18.5)	152 (16.7)	312 (17.6)	91 (18.5)	89 (17.5)
Not depressed *(n [%])*	705 (81.5)	756 (83.3)	1461 (82.4)	400 (81.5)	398 (82.5)
CES-D *(mean [SD])*	9.0 (9.2)	8.6 (7.9)	8.8 (8.6)	8.9 (9.4)	8.7 (8.0)

	Stress Study I	Stress Study II	Sample in pooled cross-section	Sample in longitudinal analysis	
				At Stress I	At Stress II
Anxiety *(mean [SD])*	15.7 (5.1)	15.6 (4.8)	15.6 (5.0)	15.7 (5.2)	16.0 (5.1)
Chronic burden *(mean [SD])*	1.2 (1.2)	1.2 (1.2)	1.2 (1.2)	1.2 (1.3)	1.2 (1.2)
Emotional support *(mean [SD])*	27.1 (6.0)	27.2 (6.5)	27.2 (6.3)	27.4 (5.9)	27.2 (6.7)

[a] Participants on HRT or steroids or who were missing long-term air pollution exposures were excluded.

[b] Category not shown is white.

[c] Category not shown is at least a college degree.

[d] Category not shown is non-smoker.

[e] Score of 16 or more on CES-D considered depressed.

Table 2.

Mean (standard deviation) and sample size of long-term air pollution exposures and cortisol in MESA Stress Studies

	Stress I	Stress II	Sample in pooled cross-section	Sample in longitudinal analysis	
				At Stress I	At Stress II
Annual average air pollution concentrations					
Ambient $PM_{2.5}$ ($\mu g/m^3$)	16.2 (2.1)	11.7 (1.7)	13.9 (3.0)	16.1 (2.1)	12.2 (1.5)
Individual $PM_{2.5}$ ($\mu g/m^3$)	12.3 (2.1)	8.5 (2.0)	10.3 (2.8)	12.3 (2.1)	9.2 (1.5)
NO_x (ppb)	64.9 (17.5)	38.5 (16.6)	51.2 (21.5)	65.4 (17.8)	44.5 (13.7)
NO_2 (ppb)	28.2 (4.8)	20.5 (7.5)	24.2 (7.4)	28.3 (4.9)	23.8 (5.1)
Black Carbon (10^{-5}/m)	1.3 (0.2)	1.1 (0.3)	1.2 (0.3)	1.3 (0.2)	1.3 (0.2)
Cortisol					
N persons[a]	*867*	*926*	*1793*	*491*	*491*
N days[b]	*2573*	*1847*	*4420*	*1467*	*978*
N samples[c]	*14646*	*13811*	*28457*	*8422*	*7365*
Average salivary cortisol (nmol/L)			10.3 (10.6)	9.1 (9.2)	10.9 (10.9)
Wake up cortisol (nmol/L)			15.4 (10.9)	13.4 (9.2)	16.8 (10.7)
Wake-to-bed slope (mean (SE))			−0.83 (0.01)	−0.91 (0.02)	−0.77 (0.02)
Area under the curve (mean (SE))			1.7 (0.02)	1.5 (0.2)	1.7 (0.3)

[a] Participants on hormone replacement therapy, inhaled or oral steroids or missing air pollution predictions were excluded.

[b] Days with no time since wake-up recorded for the entire day or no cortisol values for the entire day were excluded.

[c] Samples with missing cortisol values, time sample was taken, or unreliable cortisol values (>100 or 0 nmol/L) were excluded.

Table 3.

Pooled cross-sectional association between long term air pollution and salivary cortisol[a]

Pollutant	% difference at wake-up	% difference in CAR[b]	% difference in early decline	% difference in late decline	% difference at bedtime	% difference in AUC	% difference in wake-to-bed slope
Ambient $PM_{2.5}$ (5 μg/m^3)	−3.40 (−13.45, 5.75)	0.51 (−7.32, 9.00)	−3.44 (−9.82, 2.57)	0.58 (−0.21, 1.37)	0.19 (−9.56, 10.99)	−4.06 (−11.81, 3.16)	0.31 (−0.36, 0.98)
Individual $PM_{2.5}$ (5 μg/m^3)	−4.43 (−13.83, 4.19)	1.19 (−6.28, 9.26)	−0.22 (−6.46, 5.65)	0.64 (−0.14, 1.43)	5.64 (−4.66, 17.06)	0.45 (−6.40, 7.79)	0.60 (−0.07, 1.27)
NO_x (40 ppb)	5.92 (−4.46, 17.43)	−1.15 (11.31, 8.10)	−1.63 (−8.93, 5.17)	0.55 (−0.35, 1.45)	10.29 (−1.85, 23.93)	5.89 (−1.76, 14.13)	0.36 (−0.41, 1.15)
NO_2 (10 ppb)	9.67 (−0.16, 20.47)	−1.62 (−9.83, 5.98)	−4.21 (−10.65, 1.86)	0.14 (−0.63, 0.92)	3.48 (−5.56, 13.38)	2.86 (−3.28, 9.39)	−0.17 (−0.81, 0.46)
Black Carbon (0.5 $10^{-5}m^{-1}$)	7.12 (−2.46, 17.64)	−2.37 (−10.51, 5.17)	−2.94 (−8.70, 2.51)	0.37 (−0.38, 1.12)	5.50 (−3.92, 15.84)	2.86 (−3.45, 9.58)	0.05 (−0.57, 0.68)

[a]Coefficients and 95% confidence intervals presented as percent differences (because cortisol was log transformed) per increment of air pollutant (i.e. 5 μg/m^3 higher $PM_{2.5}$, 40 ppb higher NO_x, 10 ppb higher NO_2 and 0.5 $10^{-5}m^{-1}$ higher black carbon). Model is adjusted for age (continuous), race/ethnicity, gender, study site, and wake-up time, education, employment status, income-wealth index, and neighborhood deprivation index. Model 1 and Model 3 (minimally and more fully adjusted models) can be found in supplementary materials.

[b]CAR: Cortisol awakening response, the slope from wake-up to 30 minutes after wake-up

[c]AUC: Area under the curve

Table 4.

Longitudinal associations between long-term air pollution and salivary cortisol[a]

Pollutant	Wake-up	CAR[b]	Early decline	Late decline	Bedtime	AUC[c]	Wake-to-bed slope
Mean (SE) 5-yr change[d]	0.19 (0.11, 0.26)	−0.16 (−0.24, − 0.09)	0.05 (0.00, 0.10)	0.01 (0.01, 0.02)	0.29 (0.22, 0.36)	0.18 (0.12, 0.24)	0.01 (0.004, 0.013)
Ambient $PM_{2.5}$ (5 $\mu g/m^3$)	0.10 (−0.15, 0.35)	−0.01 (−0.33, 0.31)	−0.02 (−0.22, 0.19)	0.00 (−0.02, 0.03)	0.09 (−0.07, 0.25)	0.13 (−0.07, 0.32)	0.002 (−0.012, 0.016)
Individual $PM_{2.5}$ (5 $\mu g/m^3$)	−0.03 (−0.24, 0.19)	0.02 (−0.24, 0.29)	0.06 (−0.11, 0.24)	0.00 (−0.02, 0.02)	0.07 (−0.09, 0.23)	0.06 (−0.14, 0.27)	0.003 (−0.011, 0.016)
NO_x (40 ppb)	0.02 (−0.28, 0.32)	−0.22 (−0.54, 0.09)	0.10 (−0.09, 0.28)	0.01 (−0.01, 0.03)	−0.02 (−0.21, 0.18)	0.06 (−0.19, 0.30)	−0.001 (−0.011, 0.009)
NO_2 (10 ppb)	0.01 (−0.19, 0.21)	−0.16 (−0.37, 0.05)	0.03 (−0.10, 0.15)	0.01 (−0.01, 0.02)	−0.07 (−0.19, 0.06)	−0.01 (−0.16, 0.14)	0.014 (0.000, 0.028)
Black Carbon (0.5 $10^{-5}m^{-1}$)	0.00 (−0.19, 0.19)	−0.14 (−0.34, 0.06)	0.05 (−0.08, 0.18)	0.00 (−0.01, 0.01)	−0.06 (−0.19, 0.07)	−0.05 (−0.20, 0.10)	0.007 (−0.003, 0.016)

[a] Coefficients and 95% confidence intervals presented as mean cortisol differences in 5-year change per increment of air pollutant (i.e. 5 $\mu g/m^3$ higher $PM_{2.5}$, 40 ppb higher NO_x, 10 ppb higher NO_2 and 0.5 $10^{-5}m^{-1}$ higher black carbon). Model is adjusted for age at Stress Study I (continuous), race/ethnicity, gender, study site, and wake-up time, education, employment status, income-wealth index, neighborhood deprivation index and time between Stress Study visits. Model 1 and Model 3 (minimally and more fully adjusted models) can be found in supplementary materials.

[b] CAR: Cortisol awakening response, the slope from wake-up to 30 minutes after wake-up

[c] AUC: Area under the curve

[d] These estimates express average change over time derived from a model without adjustment for air pollution exposures. Model is adjusted for mean age, gender, race/ethnicity, income-wealth index, day of sampling, time at wake-up centered at 700h and time between visits.