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Chronic exposure to inhaled, traffic-related nitrogen dioxide and a blunted cortisol response in adolescents

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Abstract

Background—Chronic health effects of traffic-related air pollution, like nitrogen dioxide (NO_2), are well-documented. Animal models suggested that NO_2 exposures dysregulate cortisol function.

Objectives—We evaluated the association between traffic-related NO_2 exposure and adolescent human cortisol concentrations, utilizing measures of the cortisol diurnal slope.

Methods—140 adolescents provided repeated salivary cortisol samples throughout one day. We built a land use regression model to estimate chronic NO_2 exposures based on home and school addresses. We then generated model-based estimates of the association between cortisol and NO_2 exposure one year prior to cortisol sampling, examining changes in cortisol diurnal slope. The final model was adjusted other criteria pollutants, measures of psychosocial stress, anthropometry, and other demographic and covariates.

Results—We observed a decrease in diurnal slope in cortisol for adolescents exposed to the estimated 75th percentile of ambient NO_2 (high exposure) relative to those exposed at the 25th percentile (low exposure). For a highly exposed adolescent, the log cortisol was lower by 0.06 μg/dl at waking (95% CI: −0.15, 0.02), 0.07 μg/dl at 30 minutes post waking (95% CI: −0.15,

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0.02), and higher by 0.05 μg/dl at bedtime (95% CI: 0.05, 0.15), compared to a low exposed adolescent. For an additional interquartile range of exposure, the model-based predicted diurnal slope significantly decreased by 0.12 (95% CI: −0.23, −0.01).

Conclusions—In adolescents, we found that increased, chronic exposure to NO_2 and the mixture of pollutants from traffic sources was associated with a flattened diurnal slope of cortisol, a marker of an abnormal cortisol response which we hypothesize may be a mechanism through which air pollution may affect respiratory function and asthma in adolescents.

Keywords

Air pollution; Adolescents; Epidemiology; Cortisol; HPA Axis; Neighborhood

1. Introduction

Exposure to air pollution in human studies has been consistently associated with a wide range of negative health outcomes [1,2]. Animal studies have suggested that air pollution may impact a major endocrine subsystem, the hypothalamic-pituitary-adrenal (HPA) axis, and alter the typical release of cortisol from the adrenal gland [3,4]. Dysregulation of this neuroendocrine subsystem has been associated with metabolic disorders [5], cardiovascular dysfunction [6], and neuropsychiatric disorders [7]. Cortisol is also responsible for assisting in the regulation of immune and inflammatory responses in the airways [8], but repeated, long-term exposure to high levels of cortisol may cause a counterregulatory response by the white blood cells that limits the inhibitory effects of cortisol and results in the promotion of inflammatory diseases like asthma [9]. Even though mechanistic evidence for air pollution's action on the HPA axis and cortisol has largely come from animal models [10], there is an overlap between HPA axis-related diseases and diseases that have been associated with air pollution exposure, like depression [11,12]. To date, few studies of air pollution and cortisol response in humans exist, especially in children and adolescents [13].

Cortisol is a steroid hormone produced in the adrenal gland and readily sampled from saliva [14]. Cortisol concentrations follow a diurnal rhythm where daily values peak approximately 30 to 45 minutes after waking, followed by a steady decline throughout the day. Lowest daily cortisol values typically occur overnight, with values cyclically increasing again in the hours prior to waking [14].

Due to the time-dependent nature of the measure, cortisol analyses necessarily include multiple measurements during the day. Diurnal slope, or the change in cortisol values from post-waking peak to their nighttime low point, is a frequently used measure of HPA axis function. Flattened diurnal slopes are a marker of an abnormal cortisol response and have previously been associated with chronic exposures to psychosocial stress [15]. These flattened slopes are more generally described as having a lower post-waking cortisol peak and higher end-of-day values. Additionally, flattened diurnal slopes have been identified as a superior predictor of both psychosocial stress and potential HPA axis dysregulation relative to other measures of cortisol, like total daily cortisol output ("area under the curve") or cortisol awakening response [14,16].

Here, we investigate the degree to which land use regression (LUR) modeled nitrogen dioxide (NO_2) exposure from traffic-related air pollution is associated with a flattened diurnal cortisol slope in adolescents ages 12 to 17 years who participated in the Los Angeles Family and Neighborhood Survey (LAFANS), wave 2, that contained information on self-reported psychosocial stressors, demographic information, and collected repeated saliva cortisol samples from participants.

2. Materials and Methods

2.1. Sample Population

Participants were enrolled in the Los Angeles Family and Neighborhood Survey (LAFANS), Wave 2, a population-based study in Los Angeles County, California to study the complex, multilevel influence that neighborhoods and families have on child development [17]. Data were collected in two waves, the first conducted in 2000 and 2001 and the second from 2006 through 2008. Wave 1 sampled 3,090 households from 65 census tracts. Within each household sampled, adults and children were enrolled and consented/assented for their participation in the interviews. The second wave comprised of participants who responded in the first wave and remained in their neighborhood or had moved away but could still be traced, and new entrants into the original neighborhoods. Wave 2 re-interviewed 1,091 of the original 3,140 children who participated in Wave 1 and added 296 new neighborhood entrants under the age of 18, for a total of 1,387 children.

In addition to interviewing, Wave 2 introduced health assessments for a random subset of 492 participants between 3 and 17 years of age, which included anthropometry, spirometry, and salivary cortisol measurements. Older children, between the ages of 12 and 17, were asked a more detailed battery of questions that included information on psychosocial stressors. Because both acute [18] and chronic stress [19,20] modulate the HPA axis and subsequent cortisol release, only adolescents were included in this analysis (n = 140) to allow for adequate covariate control. Data collection occurred with approval from the RAND Institutional Review Board. Subsequent data analyses were carried out with approvals from the RAND and the University of California, Los Angeles Institutional Review Boards.

2.2. Saliva Cortisol

Parents of the participating children were trained by interviewers to gather saliva samples using absorbent, cellulose-cotton tipped sorbette swabs on the end of short plastic sticks, previously identified as practically advantageous relative to other saliva collection techniques [21,22]. This method harvested more than adequate amounts of saliva for laboratory assays of cortisol, remained stable at refrigerator temperatures for a week, was comfortable for the study subject, and could be accurately carried out with minimal training.

Parents were instructed to collect samples at three time points during a single day: immediately when the adolescent woke up, 30 minutes after waking, and at bedtime. Also known as “sponge-pops”, parents placed these into the adolescent’s mouth, under their tongue for 60 seconds in order to collect an adequate amount of saliva. Subsequently, the

swabs were sealed in test tubes, stored in home refrigerators, and sent out the following day for laboratory analysis.

Participants were not allowed to provide samples if they ate or drank prior to the sample collection time point and were required to abstain from alcohol and dental work in the preceding 24 hours before the day of collection. Samples were also rejected if they were contaminated with blood or if cortisol values exceeded maximum assay sensitivity or had abnormally large intra-assay differences. Detailed information on saliva collection protocols is available from the RAND Corporation and collection device manufacturer [17,23]. The second sample must have been collected between 15 and 60 minutes after waking to be included and the third sample taken at bedtime was only included if the subject was awake for at least 10 but no longer than 20 hours.

2.3. Exposure Assessment

An LUR model to estimate annual NO_2 exposures was created for Los Angeles County using data collected over two weeks from 201 passive air samplers (part number PS-100, Ogawa & Company USA, Inc, Pompano Beach, FL) placed in the LAFANS neighborhoods during both October 2006 and February 2007. The final prediction surfaces explained 85% in the variation of NO_2 concentrations over the two weeks. Detailed information about these air pollution estimates has been published previously [24,25]. The estimated NO_2 exposure was for the one year prior to the LAFANS, wave 2 data collection date and was not adjusted for seasonality. Figure 1 displays the final prediction surface for NO_2, a marker for the mixture of pollutants from traffic sources.

In addition to the unadjusted effect of NO_2, in adjusted models we also controlled for $PM_{2.5}$ (fine particulate with aero-dynamic diameter ≤ 2.5 microns) and ozone exposure measures to isolate the role of near-source, traffic-related mixture of exposure, as represented by NO_2 in our LUR model from spatially more homogeneous, area-wide exposures. These two pollutants and our LUR traffic marker were not highly correlated in the Los Angeles region ($\rho < .70$). $PM_{2.5}$ and ozone exposure measures were generated via interpolation using a kriging algorithm with routinely collected, government ambient monitoring station data from 2002 and 2000, respectively [25]. Thus, $PM_{2.5}$ and ozone concentrations represent background levels for both pollutants and both are more homogeneously distributed across the LA basin. Air pollution exposure estimates were time-weighted for 3 locations: current home, any previous homes (within the preceding 12 months), and 1,080 hours spent at school per year.

2.4. Demographic, socioeconomic, and health characteristics

An adult household participant reported on previous year's household income. Race/ethnicity of the child was reported by the adult in the home as being White, Black, Latino, Asian, Pacific Islander, or Native American. The latter two categories were collapsed into "Other" in this analysis due to small subgroup sizes. The household's adult also reported on smokers living in the home and the use of air conditioning. A previous analysis of LAFANS wave 2 data found that less than 2% of adolescents reported smoking [27], which was deemed too low of a prevalence for inclusion in further analysis. Interviewers measured the height

and weight of participants during study visits and recorded the child's use of medications for controlling asthma.

2.5. Psychosocial Stress and Neighborhood Cohesion

Three levels of stressors were considered as covariates in this analysis: neighborhood, family, and interpersonal. As described previously [28], neighborhood-level stress was measured as a function of neighborhood cohesion, as reported by an adult living in the same household as the child participant. The adult was asked about the following scenarios: (a) this is a close–knit neighborhood, (b) there are adults kids can look up to, (c) people are willing to help their neighbors, (d) neighbors generally don't get along, (e) adults watch out that kids are safe, (f) people in neighborhood don't share same values, (g) people in neighborhood can be trusted, (h) parents in neighborhood know kid's friends, (i) adults in neighborhood know local kids, and (j) parents in neighborhood know each other. The adult further reported on: (k) neighbors would do something if kids were skipping school and hanging out on a street corner, (l) would do something if kid does graffiti, and (m) would scold kid if showing disrespect. Response options for (a) through (j) were: 1 to 5 or strongly agree to strongly disagree and responses for (k) through (m) were: 1 to 5 or very likely to very unlikely. Summary scores were computed to generate a neighborhood stress score. Neighborhood cohesion may protect against the effects of stress [29] and stronger social bonds have been associated with steeper diurnal cortisol slopes [30].

Family stress was measured as a composite of the adolescents' responses to six questions regarding the stability of their family's dynamics and relationships representing the degree to which the child experiences stressful situations in the home. Adolescents answered the following questions for the family stress score: (a) people in my family fight a lot, (b) people in my family hardly ever lose their tempers, (c) people in my family sometimes get so angry they throw things, (d) people in my family always calmly discuss problems, (e) people in my family often say mean things to each other, and (f) people in my family sometimes hit each other. Response options were: 1=True, 2=Sometimes True, and 3=Not True. Average of responses was computed to generate the family stress score. Family stress, like marital discord, has been identified as modulating cortisol level in children [31].

Furthermore, participants were asked if they experienced any of the following events during the preceding 12 months: (a) someone tried to steal something from them by force or by threatening them, (b) something was stolen from them, (c) someone tried to sell them drugs or did sell them drugs, or (d) they saw someone get shot or shot at with a gun (1=Yes, 0=No). The average of responses forms the life events stress score. Such stressful experiences have been previously described as HPA axis dysregulators in younger populations [32,33].

2.6. Statistical Analysis

We assessed the association between NO_2 exposure and the rate of change of salivary cortisol, measured as μg/dl, from daily peak (the second morning sample) to bedtime low using an unadjusted and adjusted repeated measures regression analysis (PROC MIXED; SAS 9.3, SAS Institute, Cary, NC). We used covariate-adjusted, model-based predictions to

test the estimated difference per one interquartile range (IQR) increase in NO_2 exposure and associated changes in diurnal slope. Observed saliva cortisol values, as expected, were notably right-skewed, thus a log transformation was used to normalize this outcome variable. In a sensitivity analysis, we also stratified by gender due to gender-dependent differences in cortisol reactivity to exogenous stressors [34].

Typical saliva cortisol concentrations are known to follow an “inverted J” shape with moderate wakeup values, a spike shortly after wakeup, and a slow decay throughout the remainder of the waking hours. Due to this expected non-linear shape, the modeled function of cortisol was allowed to vary at each observed time point. Therefore, time was handled nominally, with a NO_2 * time interaction term giving estimates for the effect of NO_2 exposure at each of the three time points measured. An estimated diurnal slope was calculated as the predicted difference between the post-waking cortisol peak and bedtime low. Point-wise and overall slope differences were tested using time-specific linear functions between subgroup-specific exposure quantities while covariates were fixed at their average values.

In addition to controlling for estimated $PM_{2.5}$ and ozone exposures, we adjusted for the three psychosocial stressors (neighborhood, family, and interpersonal), child’s age, height, and weight, family income, race/ethnicity, child’s use of medications to control asthma, cohabitation with cigarette smokers and use of air conditioning in the home. Cohabitating with a smoker and the filtering effect of air conditioning use could mask the role of ambient air pollution. For model fitting, we focused on comparing within-subject covariance structures. Of the possible structures modeled, antedependence presented the best fit, offering the smallest Bayesian Information Criterion and performing better than the null independence model ($\chi^2 = 25.00$, $p < .0001$).

Secondary analyses will include participants under the age of 12 to examine the relationship of NO_2 and cortisol diurnal slope without being able to control for psychosocial factors lacking in LAFANS for this younger population. Further, we will evaluate the presence of statistical interactions between NO_2 and the three psychosocial stressors.

3. Results

Table 1 presents the distribution of exposure measures, demographics, and other covariates for males and females. Among the participants with valid saliva cortisol measurements (n=140), the mean age was 14.3 years and 52% were female. The majority were Latino, and less than a quarter of participants cohabitated with a smoker. Boys were about twice as likely to use asthma medication as girls; the overall prevalence of medication use was 9%. Adolescents reported experiencing an average of one stressful event in the past 12 months. Average NO_2 annual exposures from the LUR ranged from 6.2 ppb to 34.0 ppb, with a mean of 23.5 ppb and IQR of 5.3. Salivary cortisol levels when first waking up had a median of 0.26 μg/dl and 30 minutes after waking, they reached their maximum with a median of 0.36 μg/dl; at bedtime, the median cortisol had fallen to 0.04 μg/dl (Table 1). An empirical summary plot of the log-transformed means of saliva cortisol is presented in Figure 2.

We used a linear combination of coefficients to generate model-based, covariate-adjusted, and exposure-specific estimates of diurnal cortisol slopes. Slopes were compared between hypothetical 25th and 75th percentile exposures to NO_2. There was a decrease in diurnal slope, or the bedtime low minus the 30 minutes post waking peak value, in salivary cortisol for participants exposed to the 75th percentile of ambient NO_2 relative to those exposed to the 25th percentile. For an adolescent with an estimated exposure at the 75th percentile, the log cortisol was lower by 0.06 μg/dl at waking (95% CI: −0.15, 0.02), 0.07 μg/dl at 30 min post waking (95% CI: −0.15, 0.02), and higher by 0.05 μg/dl at bedtime (95% CI: −0.05, 0.15), compared to those at the 25th percentile (Table 2).

The diurnal slope of the low exposed (the 25th percentile of estimated NO_2 exposure) was −0.93. In contrast, those with an added IQR of exposure to NO_2 (75th percentile) had a predicted diurnal slope of −0.81, and the difference in slopes, 0.12, was statistically significant at alpha < .05 (95% CI: −0.23, −0.01). The model-based estimation results are presented in Table 2 and plotted in Figure 3. Table 2 also displays the model-based estimation results for an IQR difference of both past year $PM_{2.5}$ and ozone exposure on cortisol diurnal slope. The predicted difference in diurnal slope for those exposed to the 75th vs 25th percentile of $PM_{2.5}$ was −0.04 (95% CI: −0.10, 0.02) and for those exposed to the 75th vs 25th percentile of ozone was 0.05 (95% CI: −0.09, 0.18). Decreased waking cortisol was associated with an added IQR of ozone exposure (0.05, 95% CI: −0.04, 0.13).

In an unadjusted model, the crude association of a flattened diurnal slope was also significant (95% CI: −0.20, −0.03) with a difference in slopes of −0.11. The change in estimate was approximately 4%, suggesting that controlling for the covariates strengthened the associations. In a sensitivity analysis, when stratifying the analysis by gender, we found that males had a larger difference in slopes compared to females (−0.21, 95% CI: −0.46, 0.03). Further, we examined different time-weighted exposure estimates for NO_2 exposure and found that the difference in average one-year NO_2 exposures time-weighted for school attendance (used in this analysis) was 2% larger than and home-only estimates.

In secondary analyses, we included interaction terms between NO_2 exposure and the three types of psychosocial stressors to identify potential synergistic associations. However, for family, neighborhood, and personal stressors, no statistically significant interaction was detected (p = 0.50, 0.31, and 0.41, respectively). We also evaluated the estimated effect of past year NO_2 exposure on diurnal cortisol slope among all participants less than 12 years of age with valid cortisol samples. However, for these children, we were not able to control for psychosocial stressors (family stress and stressful events) available for older participants and only included measures of neighborhood cohesion reported by an adult in the home. Model-based predictions indicated that there was no statistically significant difference in younger children modeled as exposed to the 75th percentile of NO_2 relative to those exposed at the 25th percentile (−0.02, 95% CI: −0.16, 0.11; data not shown).

4. Discussion

We found that past year NO_2 exposures as indicators of traffic-related air pollution, derived from a spatially well-defined LUR model, were associated with blunted diurnal slopes of

salivary cortisol in adolescents living in Los Angles. Using LAFANS interview data for subjects with three saliva collections over one day and annual average traffic-related air pollution at home and school, we for the first time report associations in adolescent humans that suggest a blunted cortisol response with exposure to traffic-related air pollution.

Previously, animal models consistently suggested that long-term exposure to inhaled air pollution can lead to chronic activation and dysregulation of the HPA axis leading to glucocorticoid resistance [4]. Inhaled particulate matter increases corticosterone levels in adult rats relative to controls [3], while repeated ozone exposure not only increased corticosterone levels but induced antisocial behavior [10]. Nitric oxide (NO) and NO_2, are markers of the mixture of pollutants from automobile traffic exhaust [35]. These component gases have been associated with changes in cortisol. In sheep, exogenous NO exposure was associated with the inhibition of cortisol production [36]. In a series of animal studies of dogs, guinea pigs, rats, mice, and rabbits, NO_2 exposure strained the pituitary and adrenal glands, which diminished cortisol availability [37]. Our results of flatter diurnal slopes associated with higher NO_2 exposures add to the existing literature [38,39] by suggesting that ambient traffic-related air pollution may have the potential to disrupt components of the endocrine system in human adolescents. If replicated, these findings may be especially relevant for younger populations as they might be more heavily exposed to air pollution while spending time outdoors due to their higher breathing rates and outdoor physical activities [40]. However, we did not find a flattened diurnal slope with either $PM_{2.5}$ or ozone exposures. While the $PM_{2.5}$ association was in the same direction as NO_2, it was of smaller magnitude and did not reach statistical significance, despite the aforementioned previous evidence for its role in cortisol response modulation. One possible explanation for our null result might be that our exposure estimates for NO_2 are much better indicators of localized exposure to the mixture of traffic-related air pollutants than the kriged $PM_{2.5}$ surface generated from government monitors. This might point to the greater relative importance of traffic-related air pollutants on the cortisol response compared with other sources in Los Angeles. Ozone is generally negatively associated with modelled NO_2 and traffic in in the LAFANS neighborhoods.

Acute stress and acute exposure to air pollution have been shown to activate the HPA axis and stimulate the release of cortisol [41,42]. However, chronic exposure to stress and overactivation of the HPA axis results in diminished cortisol responses over time, a status known as hypocortisolism [43,44]. Chronic HPA axis activation in humans can result in long-term blunting of cortisol profiles where the morning peak values are lower and nighttime values are higher than normal [45]. A similarly flattened diurnal slope was observed in our participants exposed to increased concentrations of NO_2 while controlling for multiple types of psychosocial stressors.

Associations between cortisol profiles and NO_2 exposure were somewhat stronger in males. This may be a chance finding or could be attributed to higher exposure to air pollution in male adolescents who may spend more time outside exercising. In LAFANS, boys reported playing sports as a social activity 35% more frequently than girls ($p = 0.001$). Boys also reported engaging in vigorous exercise 21% more frequently than girls. However, our LUR model-based NO_2 exposures for boys were only slightly (0.17 ppb) higher than for girls, but

their higher physical activity rates might have resulted in the inhalation of more air pollutants.

We restricted our analysis to adolescents ages 12 through 17. Because psychosocial stress is a known activator of the HPA axis [18–20] and is associated with socioeconomic factors that are linked to air pollution exposure [46], adequate confounder control for stress was necessary. For children between the ages of 3 and 11, LAFANS collected limited information on psychosocial stressors even though salivary cortisol was sampled in the same manner as for the adolescents. The results of a diminished association between NO_2 and diurnal cortisol slopes in these younger children may suggest the influence of uncontrolled confounding due to unmeasured psychosocial stress. Alternatively, there is evidence that infants and younger children are less responsive to external stimuli; a neuroprotective response that fades with increasing age [47]. It is also possible that the association between NO_2 exposure and cortisol diurnal slope in younger children was masked by the age-specific and rapid development of their neuroendocrine system. Similarly, we did not find a statistical interaction between NO_2 exposure and psychosocial stress on cortisol diurnal slope even though previous research reported this for adolescents [48]. Our analysis may have been underpowered to detect this association.

In a sensitivity analysis, evaluating different time-weighted exposure estimates for NO_2 exposure, we found that the difference in average one-year NO_2 exposures time-weighted for school attendance (used in this analysis) and home-only estimates was minimal. Using home-only estimates of NO_2, the change in cortisol slope coefficient in the adjusted model was only 2% smaller, indicating that home-only and school-weighted estimates of NO_2 exposure were very similar and that the time-weighted exposure estimate was sufficiently robust.

Our findings are noteworthy since they point towards a mechanism in which air pollution may affect respiratory function and asthma in adolescents; i.e. the blunting of the cortisol response due to chronic exposure to traffic-related air pollution. In terms of childhood asthma, some explanations have been that asthma is exacerbated by air pollutants as a result of damage to the epithelial tissue in the respiratory system [49]. Specifically, NO_2 has been implicated as leading to oxidative stress and the infiltration of inflammatory cells into the lungs [50] and asthma exacerbation [30,51]. In contrast to physical damage, if NO_2 has the ability to modulate the cortisol response, this offers an alternative mechanism through which air pollution may cause respiratory disease and exacerbate asthma. Such a pathway has been suggested in previous work among asthmatic children, which found that a blunted cortisol response was consistently associated allergic asthma [52], which may be congruous with the theory that excess, chronic cortisol exposure leads to an impaired anti-inflammatory role of cortisol. Thus, we speculate that air pollution effects on inflammatory and respiratory diseases like asthma could be mediated by chronic adaptation of the endocrine system to such an exposure.

Our study has several strengths, including that the estimated NO_2 exposure was modeled for the year prior to collecting interview and saliva cortisol data, establishing a measure of chronic exposure prior to testing salivary cortisol. Further, the LAFANS study covered a

range of interview topics with information on several psychosocial stressor domains. Our analysis benefited from having a robust set of psychosocial covariates to assess adolescent's experiences in and outside their homes. Another strength is that the LAFANS cohort sampling scheme overrepresented poorer families. Lower socioeconomic status is associated with increased traffic-related air pollution exposures in the home in Los Angeles [28] and other North American Cities [46], thus we were able to examine associations across a wide range of NO_2 exposures.

A limitation of our study is that the three cortisol samples were taken only on one study day. To reduce within-subject variation of saliva cortisol levels across different days, samples should be collected on multiple days with a greater number of samples taken on each day. Studies indicated that four to six saliva collections over two to three study days are needed to minimize within-subject variance [14], though these suggestions came from studies focused on older populations [53]. In the LAFANS study, parents of participants were trained by interviewers and were provided with timers to ensure saliva collection happened at specific times. Lacking multi-day cortisol or more comprehensive daily data resulted in the analysis strategy we used; i.e. using nominal time points (waking and bedtime) instead of clock time (i.e. 8:00 am or 10:15 pm). Since we only had access to one day of cortisol data, we had to assume that the collection day represented a typical circadian cycle for the participant and that associated flattened diurnal slopes reflected long-term dysregulation instead of acute modification. Cortisol was the only relevant endocrine biomarker collected for this analysis. Future studies on this topic would benefit greatly from additional biomarker sampling of the HPA axis, like corticotropin releasing hormone or adenocorticotropic hormone, to make broader conclusions about the role of chronic air pollution exposure on neuroendocrine functioning. Further, other non-traffic-related sources of NO_2 are not accounted for in our LUR exposure model. But, due to the great relative importance of traffic-related air pollution on human health in Los Angeles relative to other cities [54], this likely played only a minor role. Lastly, psychosocial stressor information was collected at the same time as saliva cortisol samples, thus we have to assume that the stress measures reflect past and chronic stressors.

5. Conclusion

A blunted cortisol response is associated with a wide variety of serious short- and long-term adverse health outcomes. While psychosocial stress has previously been linked to changes in normal cortisol patterns, recent work in animals suggested that air pollution exposure has similar effects. Our findings corroborate these conclusions, suggesting that chronic exposure to ambient NO_2 may flatten the diurnal salivary cortisol slopes in adolescents. This points towards an important mechanism through which traffic-related air pollution may impact human health and it warrants future studies of the influence of traffic pollutants on the neuroendocrine system.

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Highlights

- Evidence of blunted cortisol response in adolescents due to chronic NO_2 exposure.
- Confounding control with psychosocial data, used to adjust for relevant stressors.
- Suggests mechanism for which air pollution affects respiratory function.
- Relevant for younger populations that spend time outdoors being physically active.

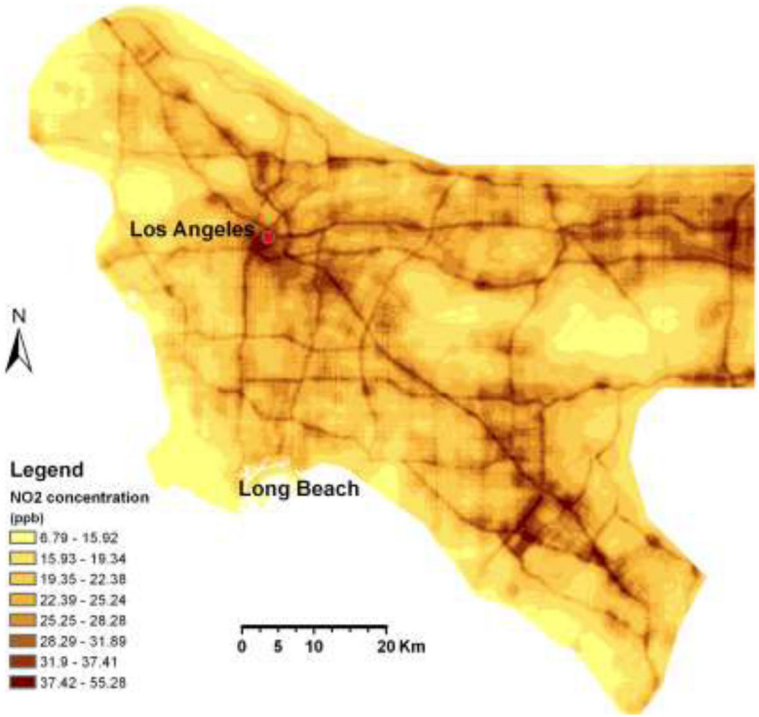

Figure 1.

Model prediction surface of NO_2 in Los Angeles County and surrounding region using the LUR method [26]

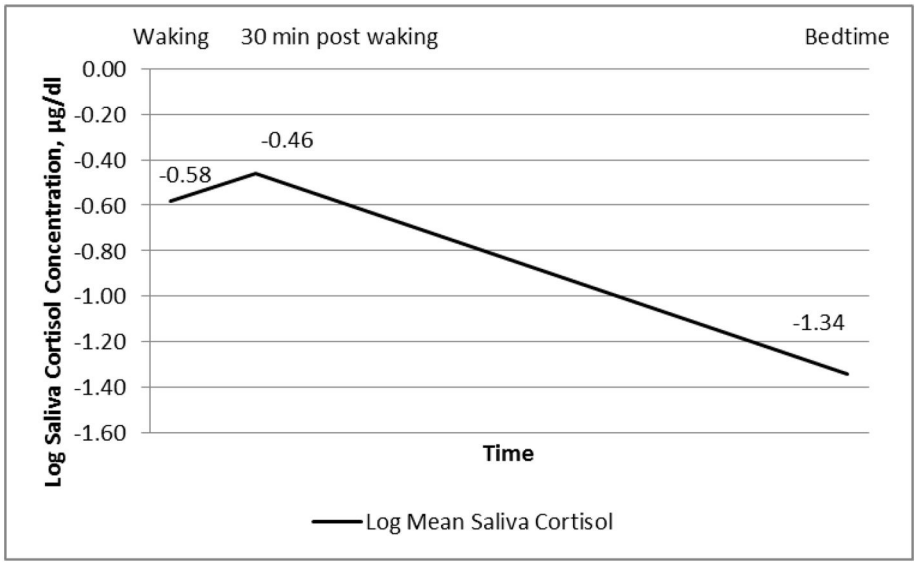

Figure 2.

Empirical mean summary plot of log saliva cortisol. Average cortisol profiles of participants achieved an expected “inverted J”-shaped curve.

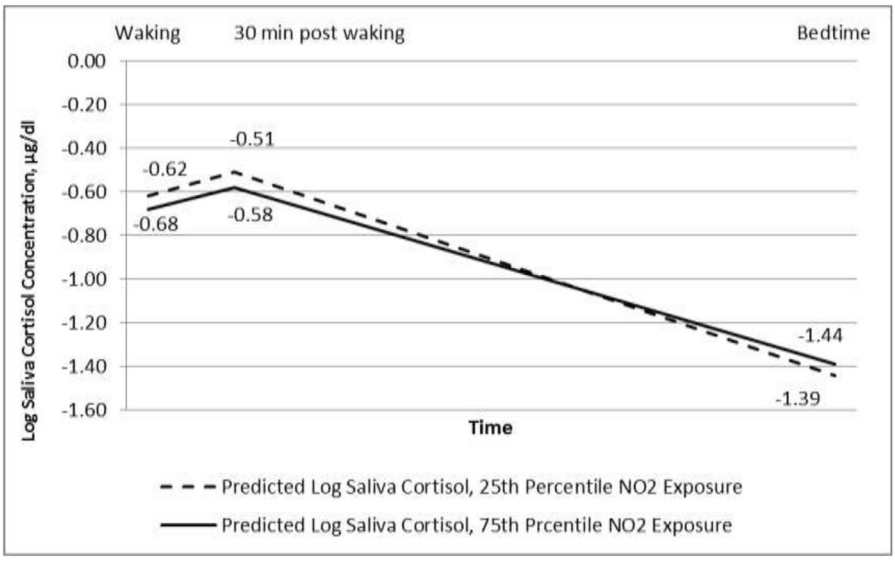

Figure 3.

Model-based predictions of log saliva cortisol by NO2 exposure in adolescents. Those exposed at the 75th percentile have a significant reduction in their diurnal slope, relative to those exposed at the 25th percentile.

Table 1

Descriptive and demographic data for participants ages 12–17 in the L.A.FANS-2 neighborhood and household survey in Los Angeles, CA, 2006–8 (n=140). Ranges -- Age: 12–17; BMI: 15.9–44.6; Annual Family Income: $128–$258,500; Saliva Cortisol, Waking: 0.02 μg/dl-1.31 μg/dl; Saliva Cortisol, 30 min. Post-Waking: 0.03μg/dl-1.34μg/dl; Saliva Cortisol, Bedtime: 0.003μg/dl-0.40μg/dl; NO_2: 6.6ppb-32.1ppb; $PM_{2.5}$: 9.0μg/m^3-23.5μg/m^3; Ozone: 46.6ppb-130.2ppb; Stressful Events: 0–3, Family Stress Score: 8–18, Neighborhood Cohesion Score: 1.2–3.9.

	Female (n = 73)	**Male (n = 67)**
Adolescent covariates		
Age, mean (sd)	14.2 (1.6)	14.4 (1.8)
BMI, mean (sd)	24.7 (6.5)	24.6 (5.7)
Asthma Medication User	3%	10%
Race		
Latino	69%	69%
White	15%	16%
Black	3%	2%
Asian	5%	0%
Other	8%	13%
Household covariates		
Cohabitates with smoker	22%	24%
Air Conditioning in the Home	47%	49%
Annual Family Income, median (sd)	$65,355 ($55,351)	$60,822 ($58,591)
Saliva Cortisol (μg/dl),		
At waking, mean (sd)	0.34 (0.24)	0.31 (0.18)
At 30 min post waking, mean (sd)	0.48 (0.29)	0.39 (0.23)
At bedtime, mean (sd)	0.08 (0.11)	0.07 (0.14)
Air Pollution Annual Exposure Estimates		
NO_2 (ppb), mean (sd)	23.1 (4.8)	23.0 (3.6)
$PM_{2.5}$ (μg/m^3), mean (sd)	20.7 (2.6)	20.4 (2.9)
Ozone (ppb), mean (sd)	74.1 (19.6)	78.2 (21.2)
Psychosocial Stressors		
Stressful events, past 12 months, mean (sd)	0.43 (0.72)	0.60 (0.96)
Family stress score, mean (sd)	14.3 (1.8)	13.5 (3.1)
Neighborhood cohesion score, percent above LAFANS median	36%	34%

Table 2

Model-based Estimation & Tests of Diurnal Slopes, Log Saliva Cortisol in adolescents, ages 12–17 years for past year NO_2, $PM_{2.5}$, and Ozone exposures. Model is adjusted for PM2.5 and ozone exposures, three psychosocial stressors (neighborhood, family, and interpersonal), child's age, height, and weight, family income, race/ethnicity, child's asthma status, cohabitation with cigarette smokers and use of air conditioning in the home.

		Estimated Log Salivary Cortisol by Time			Test of Slopes
		Waking	**30 Min Post-Waking**	**Bedtime**	**Slope (Post-waking Peak to Bedtime)**
NO_2	25th percentile	−0.62	−0.51	−1.44	−0.93
	75th percentile	−0.68	−0.58	−1.39	−0.81
	Difference (95% CI)	**0.06 (−0.15, 0.02)**	**0.07 (−0.16, 0.02)**	**−0.05 (−0.05, .0.15)**	**−0.12 (−0.23, −0.01)**
$PM_{2.5}$	25th percentile	−0.56	−0.46	−1.38	−0.92
	75th percentile	−0.58	−0.47	−1.33	−0.86
	Difference (95% CI)	**−0.01 (−0.06, 0.03)**	**−0.01 (−0.06, 0.04)**	**0.03 (−0.03, 0.09)**	**−0.04 (−0.10, 0.02)**
Ozone	25th percentile	−0.59	−0.48	−1.35	−0.87
	75th percentile	−0.54	−0.45	−1.37	−0.91
	Difference (95% CI)	**0.05 (−0.04, 0.13)**	**0.03 (−0.07, 0.12)**	**−0.02 (−0.13, 0.09)**	**0.05 (−0.09, 0.18)**