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Heterogeneity in Pulmonary Response to a Prescribed Commute

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Heterogeneity in Pulmonary Response to a Prescribed Commute

By

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B.S.
University of Texas
2010

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Abstract

Heterogeneity in Pulmonary Response to a Prescribed Commute
By Leah Yngve

Introduction: Many people are subjected to daily exposures of vehicle-related air pollution while commuting to work or otherwise. While studies have demonstrated that pulmonary inflammation is associated with exposure to vehicle-related air pollution, variations in response due to demographic characteristics, geographic characteristics, lifestyle factors, and ambient pollution have scarcely been studied previously.

Methods: 42 study participants were recruited to participate in two 2-hour scripted commutes in Atlanta, GA between the hours of 7am and 9am. Sub-clinical health measurements assessing pulmonary inflammation, lung function, and cardiovascular function were recorded immediately before the commute, directly after the commute, and every hour for 3 hours following the commute. The estimated change in exhaled nitric oxide (eNO), an indicator of pulmonary inflammation, following the commute was assessed individually and by various demographic, geographic, lifestyle, and ambient pollution factors to examine heterogeneity in pulmonary response to a commute.

Results: Exhaled nitric oxide was significantly elevated from the baseline measurement for all measurements taken following the commute. The response was not significantly different between any of the variables examined except for the distance participants traveled to the study site. At the last time point taken 4 hours after the commute, the change in eNO was significantly different ($p=0.0229$) with a greater response in participants that traveled less than participants that traveled more prior to the study commute.

Conclusions: Across differences in physical and environmental characteristics, study participants displayed sub-clinical increases in pulmonary inflammation following a commute exposure. These results demonstrate one effect of vehicle-related air pollution that a large portion of the population is exposed to and suggest factors that may influence a difference in response.

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Introduction:

Traffic related air pollution has been shown to decrease pulmonary function in asthmatic and non-asthmatic individuals. Adar et al. (2007) examined the relationship between exhaled nitric oxide (eNO), an indicator of sub-clinical pulmonary inflammation, and diesel exhaust in an elderly population. Their results showed increases in exposure to $PM_{2.5}$, a component of traffic emission exhaust, to be associated with corresponding increases in eNO. Lung function has also shown to be affected by traffic related exposures in a study of school aged children (Brunekreef et al., 1997). In this study, children who lived within 300 meters of a motorway, compared to those who lived further were found to have decrements in lung forced vital capacity (FVC), forced expiratory volume in 1 second (FEV_1), peak expiratory flow (PEF), and forced expiratory flow (FEF). Another study showed measureable changes in lung function after short-term exposures to traffic related air pollution (McCreanor et al., 2007). Participants who conducted a scripted walk along a busy street with high traffic volume were shown to have substantially decreased FEV_1 and FVC as compared to scripted walks conducted within a park.

Obesity has been shown to modify the relationship between exposure to $PM_{2.5}$ and inflammation. Dubowsky et al. (2006) found that obesity is associated with higher levels of systemic inflammation, potentially via acute oxidative stress in response to particulate matter. This effect has also been seen among asthmatic children (Lu et al., 2013). While obesity and physical activity are usually related, to date no studies have specifically looked at whether physical activity, rather than obesity, affects the oxidative stress

response to particulate matter from air pollution. Bjork et al. (2012) found evidence that active individuals have lower levels of plasma oxidized LDL, a biomarker of oxidative stress, when compared to non-active individuals. However this study did not examine how a potential stressor would affect these levels.

In a study done by Steerenberg et al. (2001), children who attended a school near a busy road exhibited a more pronounced decrease in lung function per unit increase in air pollution than those that attended a school in a rural area. Children in the urban setting had a greater reduction in PEF and an increase in eNO following increased exposure to air pollution. This suggests that regular exposure to traffic induced air pollution may heighten the inflammatory response to air pollution components. Additionally, elevated levels of inflammatory stress biomarkers were found in individuals living closer to a highway by Brugge et al. (2013). Particularly interleukin-6 was elevated, which is a cytokine linked to exposure to particulate matter. Other studies have shown that individuals living in homes or areas within 100-200 meters of a highway are at elevated risk of exposure to highway pollutants (Brugge et al., 2013; Gan et al., 2010; Genereux et al., 2008).

As the link between traffic related air pollutants and pulmonary function is strengthened, the burden that daily exposures may have on a population's health can be better understood. With 89% of Atlanta residents commuting to work in a motor vehicle for an average of 30 minutes each way (2010 Regional Commuter Survey, Clean Air Campaign), the likelihood of daily exposures to traffic related air pollution is high for this

population. Additionally, most of the national population is exposed to traffic related air pollution with 89% of the national population commuting in a motor vehicle for an average of 25 minutes each way (US Census). Therefore it is important to understand the potential health implications of these practices.

The Atlanta Commuters' Exposures Study (ACE-1) was conducted in 2010 and 2011 to increase understanding of physiological responses to exposure to traffic related air pollution while commuting. The current analysis examines the hypothesis that heterogeneity in response of respiratory endpoints to the ACE-1 commutes can be explained by: physical characteristics (BMI, age, sex, race), geographical characteristics (residential proximity to a highway, distance traveled to the study site), lifestyle factors (physical activity behaviors, normal mode of commute, daily minutes spent commuting), and environmental factors (previous day's ambient pollution, ambient pollution during commute periods).

Methods:

Study design

Twenty asthmatic and twenty non-asthmatic Atlanta residents were recruited to conduct two scripted highway commutes in Atlanta, GA, between 7am and 9am. Participants were excluded if they had diabetes; a history of myocardial infarction; an implantable cardioverter-defibrillator or pacemaker; used digoxin or beta blockers for the treatment of hypertension or cardiac arrhythmias; had a non-asthma pulmonary disease such as COPD, emphysema, or any type of lung cancer; or had a forced expiratory volume in 1 second (FEV1) less than 70% predicted at baseline. This study was approved by the Emory institutional review board and written informed consent was provided by all participants.

The routes were consistent for each commute. Research assistants monitored the route, equipment, and conditions as the only passengers. Sub-clinical health measurements were taken at Emory University immediately before, after, and at each hour for 3 hours after the commute. In-cabin air quality was measured throughout the commute.

Health Measurements and Participant Characteristics

Spirometry, exhaled nitric oxide, exhaled breath condensate, electrocardiogram, heart rate, heart rate variability, blood pressure, and blood oxygen level were measured before the commute, immediately after the commute, and every hour for three hours after the commute by a trained phlebotomist and technicians. An OHD KoKo spirometer (Occupational Health Dynamics, Birmingham, AL, USA) was used to measure forced

expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC); a NIOX MINO instrument (Aerocrine, New Providence, NJ, USA) was used to measure exhaled nitric oxide; a standardized breath-condensate collector (RTube, Austin, TX, USA) was used to collect exhaled breath condensate for analysis of malondialdehyde; Alere Cholestech LDX system (Inverness Medical, Hayward, CA, USA) was used to measure C-reactive protein (CRP); Ambulo 2400 ABPM System (Tiba medical, Portland, OR, USA) was used to measure blood pressure; a Cortisol EIA kit (ENZO life sciences Farmingdale, NY, USA) was used to measure salivary cortisol levels to detect stress; and a 5-lead Holter monitor (2010 Plus Philips Healthcare, Eindhoven, Netherlands) was used to measure heart rate variability. For this analysis, only the effect of the commutes on measured eNO levels as well as potential effect modification was considered.

Additional information was collected on each subject by a trained technician including age, sex, height, weight, race, and ethnicity. After one or both of the commutes, each participant filled out a survey to gather information including home address, workplace address, and weekly moderate and vigorous exercise behaviors. Information on daily commute characteristics were collected such as the normal mode of transportation (personal vehicle, car/van-pool, public transit, bicycle, or walk), minutes spent commuting to work, time on an interstate, and which interstate. After approximately half (34) of the commutes, an additional protocol was introduced that included questions regarding participant perception of the level of traffic, stress, and noise during the commute. Where multiple surveys were available for a given participant, the survey data

from their first commute was used to maintain consistency in data accuracy between participants.

Physical Activity

Participants were asked to indicate the number of days per week and minutes per day they participate in moderate physical activity and vigorous physical activity. According to the U.S. Department of Health and Human Services 2008 Physical Activity Guidelines for Americans adults should do at least 150 minutes of moderate physical activity, 75 minutes of vigorous activity each week, or a combination of moderate and vigorous physical activity. Therefore in order to determine if participants meet the physical activity requirement their weekly activity was summed by doubling the vigorous activity and adding it to moderate activity. Those who did not meet the 150 minutes per week requirement were categorized as "inactive" and those who exercised for at least 150 minutes per week were categorized as "active".

Exposure Measurements

Specific components of the in-cabin air quality were measured throughout the commute, however, for the purpose of this analysis the commute was used as the exclusive metric of exposure. Before the commute baseline health measurements were taken of each participant and health measurements were taken at four intervals post-exposure. Additionally, the previous day and concurrent average hourly ambient $PM_{2.5}$ and O_3 concentrations were compiled from the Georgia Department of Nature Resources' Ambient Monitoring Program Database. The difference between the average hourly

recorded concentration of $PM_{2.5}$ in the car during the commute and the average hourly ambient $PM_{2.5}$ from the previous day or concurrent time was determined as an exploration of a priming effect that current or immediately previous exposures may have had.

Living near major roads and highways may also influence exposure to traffic related air pollution (Bayer-Oglesby et al., 2006; Brugge et al., 2013; Brunekreef et al., 1997; Gan et al., 2010; Genereux et al., 2008). Therefore the linear distances of each participant's home address to major roadways and highways were calculated using ArcGIS (Environmental Systems Research Institute, Redlands, CA) and road data from the Atlanta Regional Commission. If study participants lived 200 meters or fewer from a highway or major road, they were categorized as exposed to traffic related air pollution at their residence. Those living greater than 200 meters from a highway or major road were categorized as unexposed to traffic related air pollution at their residence. This cut off was determined based off of standards in the literature and evidence that the concentrations of traffic related pollutants are no longer predictably detected beyond 200 meters of a major roadway (Levy et al., 2010).

The network (road) distance from each participant's home address to the study site at Emory University was calculated to determine how far participants traveled prior to the start of their commute session. A network analysis in ArcGIS was used to calculate the distance.

Statistical Analysis

This analysis examined the effect of the commute as the exposure on eNO for a period of four hours after the commute compared to the pre-commute baseline. Exhaled nitric oxide was distributed log-normally and therefore log-transformed prior to the analysis. The two primary approaches for assessing heterogeneity were through stratified models and unstratified models with interaction terms between the variable of interest and time variables. Heterogeneity in the association between the commute and change in eNO was examined based on physical characteristics (age, sex, race, weight status), geographical data (residential proximity to a highway or major road, distance traveled to study site), lifestyle factors (physical activity, daily minutes spent commuting, normal mode of commute) and environmental factors (previous day's ambient $PM_{2.5}$ and O_3, $PM_{2.5}$ and O_3 concentrations during commute periods, difference between car $PM_{2.5}$ measures and ambient measures). Continuous variables were categorized as follows: age – tertiles and quartiles; distance traveled prior to the commute – median, tertiles, and quartiles; daily minutes spent commuting – median, tertiles; and previous day's ambient $PM_{2.5}$ and O_3, $PM_{2.5}$ and O_3 concentrations during commute periods, difference between car $PM_{2.5}$ measures and ambient measures – median, tertiles. Where variables were grouped into tertiles, the first tertile represented the lowest values and the third tertile the highest. All other variables were binary or categorized into two categories as follows: race – Caucasian or not Caucasian; weight status – body mass index (BMI) below 25 was considered healthy weight, 25 or greater was overweight (Centers for Disease Control and Prevention, 2011); residential proximity to a highway or major road – 200 meters or less was exposed, greater was unexposed; physical activity – 150 minutes or more of

equivalent physical activity (see previous section) was active, less was inactive; and normal mode of commute – personal vehicle, car/vanpool, and public transit were grouped into a motorized category and walking or biking were grouped as non-motorized.

Mixed effect linear regression models were used to determine the association between changes within subjects in eNO following exposure to a highway commute. The model was stratified by each of the variables of interest individually to qualitatively assess differences. An interaction model was also created to determine if changes in eNO differed significantly at any time point between strata. The basic and interaction term models can be expressed as:

Basic: $\ln Y_{ijkl} = \beta_0 + b_{0i} + \sum^{j=1,4} \beta_j\ (time)_j + \gamma\ commute_k + \gamma\ strata_l$

Interaction: $\ln Y_{ijkl} = \beta_0 + b_{0i} + \sum^{j=1,4} \beta_j\ (time)_j + \gamma\ commute_k + \gamma\ strata_l + \sum^{j=1,4} \delta_{jl}\ (time_j \times strata_l) + \delta_{kl}(commute_k \times strata_l)$

where Y_{ijkl} is the natural log of eNO for subject *i* on commute *k* (first or second) at time *j* in strata *l*. β_0 and b_{0i} are the intercept and random intercept for subject *i* respectively. Time, *j*, indicates the time point immediately after the commute and each hour for the next three hours. The estimate of effect, β_j, is interpreted as the change of the outcome at time *j* from the baseline. Percent change in eNO was calculated by $(\exp(\beta_j)-1)*100$. The model assumes a spatial power covariance structure between the outcome, eNO, at different times within a subject, and variance component between-subject variance covariance structure.

In the interaction term model, the categorical strata variable was multiplied by each time variable (t1-t4) and the commute. Interaction at each time point was evaluated separately as well as the interaction with commute. The coefficient δ_{jl} represents the amount by which the overall effect on eNO is modified by the strata variable. The δ_{kl} coefficient represents the difference in effect on eNO by either the first or second commute, essentially comparing the baseline values for the first or second commute by the strata variable.

Results:

The study included 42 subjects who participated in two commutes each for a total of 80 commutes. By study design, 21 of the subjects were asthmatics and 21 were non-asthmatics. The ages ranged from 20 to 59, half (21) were male, and 26 were Caucasian. The cohort was generally healthy with the majority (31) of a healthy weight and physically active (32) (Table 1).

Stratified and Interaction Results

Using the mixed effects linear regression model for the cohort as a whole, exhaled nitric oxide (eNO) changed significantly from the pre-commute baseline to each post-commute measurement (p-values ranged from <0.0001 to 0.0003) (Table 2, Figure 1). Results from the stratified analysis revealed that significant change in eNO from baseline was preserved for most strata. Where possible for continuous data, results are presented for tertile comparisons to preserve sample sizes.

Age

Age was stratified into tertiles and significant change in eNO from baseline was observed for time points in each tertile. In the youngest tertile, change in eNO at all times (t1-t4) was significantly elevated from the baseline (p=[<0.0001, 0.0008), while only t1-t3 were significant for the middle and older tertiles (p=[0.008, 0.08]; p=[0.003, 0.13] respectively). When age was further examined for interaction to determine if change in eNO differed significantly between the age groups, no significant change by age was found.

Sex

For each sex evaluated separately the increase in eNO from baseline was significant at each post-commute time point. Though the findings show a greater increase in eNO at each time point for males compared to females, modification of response by sex at each time point was not found to be statistically significant when interaction terms of sex and time were evaluated. However, interaction of sex with the commute variable was statistically significant in models that included time and commute product terms with sex (p=0.02) (Table 3).

Weight Status

Weight status (healthy or overweight), when evaluated separately resulted in a significant increase in eNO from baseline only for the healthy weight group (p=[<0.0001, 0.0002]). The change in eNO appears to be less pronounced for the overweight group (p=[0.058,

0.89]), and the interaction between change in eNO by weight status was shown to be marginally significant at the last time point 4 hours post-commute (p=0.053) (Table 3).

Race

The estimates of change in eNO from baseline for Caucasian study participants and non-Caucasian participants were statistically different from baseline for all time points. Non-Caucasians had greater elevation in eNO post-commute than Caucasians at all time points, however, the interaction was not statistically significant likely due to the small, predominately Caucasian cohort.

Physical Activity

When physical activity was assessed, the estimates for elevation in eNO from baseline were significant for the physically active category (p=[<0.0001, 0.0012]), which was greater at each time point than the estimates for the inactive category. The estimates for change in eNO in the inactive category were not significantly elevated from the baseline value (p=[0.12, 0.86]). No significant interaction was found at any time point.

Highway/Major Road Exposure

All estimates for the change in eNO from baseline for the non-exposed group were significantly elevated (p=[<0.0001, 0.0046]), while just at t2 and t3 were the estimates significantly higher than the baseline for the highway or major road-exposed group (p=0.0963, 0.0051, 0.0195, 0.1217 for t1-t4). Interaction for change in eNO by exposure group was not statistically significant.

Commute Mode

For both commuters using motor vehicles to get to work and those that walk or bike, estimates for change in eNO were significant at all time points. Estimates for change in eNO for non-motorized commuters were higher than those for motorized commuters; however, no statistically significant interaction was found.

Distance to Study Site

The distance traveled to the study site was broken into tertiles. Estimates of change in eNO from the baseline at all time points were significantly elevated for the lower two tertiles (1^{st} tertile % change=[11, 17]; p=[<0.0001, 0.0024]), while none of the estimates for the highest tertile were significantly different from the baseline (3^{rd} tertile % change=[-3, 6]; p=[0.13, 0.63]) (Table 2). Change in eNO for the highest tertile appeared to be lower than the other two and statistically significant interaction was seen between the groups at t4 (p= 0.023). Additionally, at t3, the lowest tertile was significantly different than the highest (p= 0.043) (Table 3, Figure 2).

Average Daily Minutes Spent Commuting

Estimates for change in eNO compared to the baseline were significant for all time points at the lowest tertile, t1-t3 in the middle tertile, and t1-t2 in the highest tertile. Interaction was not significant at any time point.

Previous 24 Hours Average Ambient $PM_{2.5}$

The average ambient $PM_{2.5}$ for the previous 24 hours was assessed by categorization into tertiles. Estimates for change in eNO compared to the baseline were significantly different from the baseline at each time point for the lower two tertiles, while only the estimates for t1-t3 were significant in the highest tertile. Though the interaction was not statistically significant, the estimates appeared to decrease as the previous day's average $PM_{2.5}$ tertiles increased.

Previous 24 Hours Average Ambient O_3

The average ambient O_3 for the previous 24 hours was assessed by categorizing the levels into tertiles. Estimates for change in eNO compared to the baseline were significant at each time point for the lower two tertiles and significant at t1 and t2 for the greatest tertile. The highest tertile appeared to have lower estimates than the lower two, however, interaction was not significant. Interaction between the previous ambient O_3 and commute number (first or second) was significant ($p=0.0098$) (Table 3).

Concurrent 2-Hour Average Ambient $PM_{2.5}$

The analysis of concurrent average ambient $PM_{2.5}$ was performed by comparing tertiles of ambient $PM_{2.5}$. At each time point, the estimates for change in eNO were significantly different than the baseline in the lower two tertiles. In the highest tertile, only the estimate for change in eNO was significant at t1. The highest tertile appeared lower than the lower tertiles, however, analysis of interaction was not significant. Interaction

between the concurrent ambient $PM_{2.5}$ and commute number (first or second) was significant (p=0.0064) (Table 3).

Concurrent 2-Hour Average Ambient O_3

In the analysis of tertiles of concurrent average ambient O_3, only the estimates for the change in eNO were significant for t1-t3 in the lowest tertile. All of the estimates for change in eNO were significant in the middle tertile, while none were significant in the highest tertile. Interaction between the tertiles for change in eNO was not significant at any time point nor with commute.

Discussion:

The results of this analysis indicate a significant difference in response to commute exposures based on the distance traveled to the study site, and suggest differences based on weight and demographics. The post-commute elevation in exhaled nitric oxide (eNO) compared to the pre-commute baseline corroborates findings from previous research (Adamkiewicz et al., 2004; Adar et al., 2007; Hussain et al., 2012; McCreanor et al., 2007; Zuurbier et al., 2011). As eNO is an indicator of pulmonary inflammation, these results suggest that a two-hour commute is sufficient to significantly increase and sustain pulmonary inflammation for at least four hours following exposure. This trend of elevated eNO following exposure was retained among many of the sub-groups studied.

Stratified Models

Physical Characteristics

Despite decreased sample sizes, stratified analyses revealed significant changes in eNO across strata. Significant estimates for change in eNO within the different sexes, Caucasians and non-Caucasians, and age tertiles demonstrate that the effect of commute exposures is consistent despite differences in physical and other socio-demographic characteristics. However, the lack of statistical interaction between these characteristics and response following the commute suggests that the response to the commute does not vary based on these characteristics. The presence of statistical interaction between sex and the first or second commute indicated that baseline levels of eNO varied by sex, though change in eNO is not significantly different between males and females. This inter-gender difference in eNO levels has been observed in other studies, however, a comparison of response by gender has not previously been reported (Adamkiewicz et al., 2004; Adar et al., 2007).

Lifestyle Characteristics

When examining variations in pulmonary inflammation following exposure to a commute, evaluation of differences across available lifestyle strata did not indicate significant differences in response. The study cohort disproportionately met recommended physical activity levels and was in a healthy weight range. Therefore, the smaller sample sizes for inactive and overweight participants (n=14, and n=22 respectively) may have contributed to a lack of precision and therefore non-significant estimates of the change in eNO from baseline. While this could be an indication that

commute related exposures do not elicit a change in eNO for inactive or overweight participants, the study may also lack sufficient power to distinguish trends at those sample sizes. In a study of youth asthmatics by Lu et al. (2013), responses to changes in $PM_{2.5}$ were assessed by weight category. Their study population had a greater representation of non-healthy weight subjects and they found that the relationship between $PM_{2.5}$ and eNO did not change by weight status. Additionally, Zuurbier et al. (2011) compared the change in eNO following 2-hour morning commutes by car, bus, or bicycle by weight group and found no differences. No studies were identified that assessed response to traffic related emissions by activity level, however, differences in baseline values have been reported for oxidative stress biomarkers (Bjork et al., 2012; Karaouzene et al., 2011). Sachs-Olsen et al. (2013) examined the relationship between physical activity and daily changes in eNO. For non-asthmatic study participants partaking in the most strenuous physical activity, eNO significantly increased as minutes of physical activity per day increased. Therefore though the relationship between physical activity and an inflammatory response to vehicle emissions is not supported by the findings from this analysis, further examination with greater power could be warranted.

The average number of minutes spent commuting on a normal workday, residential distance from a major road or highway, and normal mode of commute were all considered due to their impact on the frequency and duration of traffic related exposures which may mediate the individual response to that exposure. Biological explanations for variations in response are not currently known, though differences in response between those with different daily exposures could be due to a physical sensitivity or

acclimatization to exposure, or due to a priming effect (Magari et al., 2002). Though significant change in eNO was seen in each tertile of average minutes spent commuting, those residentially exposed and unexposed to a major road or highway, and motorized and non-motorized commute patterns, no significant differences in response were seen between the groups. Among children, significant differences in eNO have been observed for those living within an area with denser roads (Dales et al., 2008) and in an urban setting compared to suburban (Steerenberg et al., 2001), however, differences in baseline eNO by residential status were not seen in this study. Of note is that 73% of study participants lived further than 200 meters from a major road or highway and therefore the data may not have been robust enough for this examination. Despite the small sample size of non-motorized commuters (n=14), the estimates for change in eNO from the baseline were significant for this group along with the motorized group. Zuurbier et al. (2010) sampled air quality components on two-hour bicycle, car, and bus commutes and found that bicycle commuters were estimated to receive a greater amount of less concentrated pollution exposure than motorized commuters due to increased ventilation. Despite potential differences in daily exposures between motorized and non-motorized commuters, this study did not find a statistical interaction between normal commute mode and response to the scripted commute.

The on-road distance between the study site and each participant's house was calculated as a consideration of road exposure and the amount of time each study subject may have been awake before the baseline measurements were taken. The distribution of the distance to the study site was left skewed with the highest tertile more than 4 times larger

than the middle tertile and over 10 times that of the smallest tertile. The lower tertiles showed a significant change in eNO from the baseline, while the change in the third tertile was not significant. Further analysis of statistical interaction indicated that the response as a whole was different between tertiles four hours after the commute and the lowest and middle tertile were each significantly different than the highest tertile at that time. Stratified graphs of change in eNO by distance to study site tertile show a greater response in the lower tertiles than the highest.

Antosova et al. (2009) and Mattes et al. (2002) have studied the variation of eNO over the course of a day and both reported oscillation in eNO over by day with the highest levels achieved in the morning followed by a decrease after 10am. Therefore, one potential explanation for the difference in response for the study subjects traveling the furthest to the study site is a difference in daily fluctuations in eNO due to an earlier start of the day. A significant difference in baseline values of eNO was not seen between the tertiles, however a difference in the phase of the circadian rhythm could occur without values of eNO being different at the pre-commute hour.

Ambient Pollution

The previous day's 24 hour average ambient $PM_{2.5}$ and O_3 were collected for each commute and divided into tertiles for comparison. Within each tertile a significant change in eNO was observed following the commute. A difference in baseline values was assessed by testing for interaction between the previous day's pollution and the commute number. The previous day's $PM_{2.5}$ did not affect baseline values, though a statistically

significant difference was seen based on previous O_3 levels. Concurrent ambient $PM_{2.5}$ and O_3 were collected for the time period in which the commute occurred and were assessed by tertiles. The observed changes in eNO were significantly different from baseline levels for all tertiles and a difference in baseline by commute was seen just for concurrent $PM_{2.5}$. Though the effect of ambient pollution on baseline values is not the primary objective of this study, previous and current ambient pollution have been shown to affect levels of eNO, likely via sustained activation of the immune system leading to pulmonary inflammation (Adamkiewicz et al., 2004; Adar et al., 2007).

Key Findings

Visible differences in response were observed between strata, though only distance to study site was significant. One potential hypothesis to explain the observed differences is that increased exposure to pollution decreases the response mechanism following exposure to the same pollution. Study participants who traveled further to get to the study site may have received increased exposure due to the distance traveled or may generally travel long distances to work or play. This trend of a smaller response where daily exposures may be greater, though not significant, was also seen for previous and concurrent ambient $PM_{2.5}$ and O_3, and to a certain extent, for minutes spent commuting daily and residential proximity to a major road or highway. In a study of heart rate variability in boilermakers, Magari et al. (2002) found that non-smokers demonstrated a greater response to $PM_{2.5}$ than smokers did. The authors hypothesize that the routine exposure to $PM_{2.5}$ that smokers get makes them less susceptible to environmental exposure to $PM_{2.5}$. Other studies examining response to environmental exposures in

groups with differences in daily environmental exposures (Dales et al., 2008; Steerenberg et al., 2001) focused on pulmonary responses in children, which may not be an equivalent population for comparison.

Though many differences are seen between the estimates of change in eNO by strata, the majority of these differences were not statistically significant (Figure 3). This could reflect random variations within stratified groups causing a non-significant difference in estimates where a true difference does not exist, or alternatively, true differences that the study lacked power to distinguish. While many studies have examined the effect of ambient exposures and lifestyle factors on baseline levels of eNO, few have looked at how these factors influence the short-term response to an exposure. Therefore there is little in the literature to support or refute potential differences in response based on differences in physical, geographic, or lifestyle characteristics.

In the majority of the strata, a significant change in eNO from the baseline was observed for at least one of the four hours following the commute. In strata where the change in eNO was not significantly different from the baseline at all time points following the commute, the first and second time points were commonly significant while the third and particularly the fourth were more likely to not be significantly different from the baseline. This finding could be interpreted as support for a decreased effect on eNO as the amount of time following exposure increases. Where significant interaction was seen with the stratification by distance to study site, all estimates were significantly different from the highest tertile at the fourth (last) time point, whereas at the third time point just the lowest tertile was significantly different than the highest. These results suggest that the response

in eNO for the study subjects living furthest from the study site was, at the minimum, not significantly different from the baseline at the fourth time point and the lack of significance of the estimates at the previous time points suggests that the overall response for this group was marginally different from the baseline.

Similar to the distance from study site analysis, stratification by weight status yielded non-significant estimates for change in eNO at each time point for the overweight group, though estimates were significant for those of a healthy weight. Though the interaction of weight and response was not significant, at the fourth time point the p-value was nearly so at $p=0.0534$. While this is not sufficient to determine a difference in response, it is suggestive of a difference in response based on body mass index.

Though the association between eNO and asthma status is well established and frequently used to determine asthma status, the association between asthma status and short-term response to vehicle-related exposures is less clear. Previous analyses of this data did not find a significant difference in response between asthmatic and non-asthmatic subjects and therefore asthma status was not directly controlled for in this analysis. However, by nature of the mixed-effects model comparisons are first made within individuals and therefore baseline differences in eNO by asthma status will not affect the estimates of change in eNO.

Strengths

This study offers a detailed examination of how commute-related exposures affect pulmonary inflammation. The small sample size allowed for comprehensive measurements to be taken of each subject and therefore the change in pulmonary inflammation could be monitored following exposure so that lag effects could be detected. This analysis examined many factors and characteristics that have not previously been reported on and therefore offers insight into external factors that may affect pulmonary inflammation following exposure. Additionally, employment of the mixed effects linear model has the advantage that responses are first compared within subjects and therefore differences between subjects are less influential in the analysis.

Limitations

The relative homogeneity of the cohort was one limiting factor in this analysis. The study participants were mostly young adults with just 21% older than 40 and 5% older than 50. Therefore these findings may not be generalizable to a more homogenous population. Additionally, the study participants were relatively healthy; the majority had healthy BMIs and met physical activity requirements. Lack of heterogeneity was also seen in study participants' race, normal mode of commuting, and residential proximity to a major road or highway. These uneven distributions resulted in small sample sizes for some strata, which decrease the reliability of statistical tests.

Other limitations in the study are that variations in response could be influenced by factors uncontrolled for in the analysis. Exhaled nitrogen dioxide has been shown to be

affected by a circadian rhythm (Antosova et al., 2009; Mattes et al., 2002); however, it was not possible to control for this. Additionally, Ritz et al. (2011) tested the association between perceived stress, cortisol levels, and eNO. Their results indicated a significant positive association between cortisol levels following a stressor and eNO. Cortisol has been established as a biomarker indicating psychosocial stress, however their results imply that eNO is also associated with stress. Driving on congested roads in morning traffic may be stressful and therefore stress as a confounder is a large possibility. Nearly half of the study subjects were asked to rate their stress levels during the commute, but since the data was not complete, stress was not controlled for.

Finally, this analysis considered the participation in a commute as the exposure of interest; however, this inhibited the ability to account for uncontrollable differences in pollution levels between commutes.

Implications

These results indicate that across various individual characteristics an inflammatory response is elicited by commute exposures. Though the response seen was asymptomatic, short-term exposures to traffic-related $PM_{2.5}$ have consistently been associated with cardiovascular and respiratory related hospital admissions and mortality (Atkinson et al., 2014). Long-term exposure to traffic-related air pollution has been linked to increases in blood pressure and hypertension (Foraster et al., 2014), cardiopulmonary disease, respiratory disease, lung cancer, and adverse pregnancy outcomes (World Health Organization, 2010). These outcomes result from a multitude of factors, including

cumulative daily exposures to traffic-related air pollution. This study quantifies the pulmonary response seen after relatively short exposures, however, more research is needed to determine if increased daily environmental exposures decrease an individual's response to environmental insult, or if response varies by weight or physical activity levels.

Conclusions

This analysis offers an exploration of various factors that may influence pulmonary response to commute-related exposures. The study was not explicitly designed to examine many of the factors in this analysis resulting in inadequate heterogeneity and power to fully understand the impact of the variables of interest. However, the results of this analysis may provide guidance for the analyses of further studies in pulmonary response to short-term traffic-related exposures.

Clearly, traffic-related exposures have a detectable and preventable health impact. Shorter travel distances can decrease individual exposures and reducing the number of inefficient cars on roadways would both contribute to reducing air pollution. Finally, focusing on transportation systems that reduce the necessity for transport by motor vehicle can have a large impact on decreasing traffic-related air pollution.

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Table 1. Baseline characteristics of study participants.

Continuous Characteristics	**Mean**	**Median**	**SD[a]**	**Missing**
Age	33	31	10	0
Body Mass Index	23.6	23.1	3.6	0
Previous 24 hr $PM_{2.5}$ ($\mu g/m^3$)	12.8	12.5	5.9	4
Previous 24 hr O_3 (ppm[b])	0.023	0.023	0.009	4
Concurrent 2 hr $PM_{2.5}$ ($\mu g/m^3$)	14.4	14.1	7.1	5
Concurrent 2 hr O_3 (ppm)	0.018	0.018	0.010	4
Minutes Commuting (daily)	43	40	26	6
Distance to School (km)	10.0	5.4	13.4	10
eNO (ppb[c])	32	24	31	10
Categorical Characteristics		**N**		**Missing**
All commutes		84		0
Sex				0
Male		42		
Female		42		
Asthma Status				0
Asthmatic		42		
Non-Asthmatic		42		
Weight				0
Healthy Weight		62		
Overweight		22		
Race				0
Caucasian		52		
Other		32		
Meets Physical Activity Requirement				6
Yes		64		
No		14		
Residing <=200m of a Major Road				10
Yes		20		
No		54		
Commute Mode				0
Motorized Commute			70	
Non-Motorized Commute		14		

[a] SD=standard deviation, [b] parts per million, [c] parts per billion

Table 2. Effect estimates for the change in exhaled nitric oxide (eNO) from baseline unstratified and stratified by increasing distance to study site.

eNO	Estimate (x100)	SE[a] (x100)	P-value
Post-Commute	8.8	1.8	<.0001*
1 Hour after commute	11.7	2.2	<.0001*
2 Hours after commute	11.3	2.5	<.0001*
3 Hours after commute	9.9	2.7	0.0003*
Stratification by Distance to Study Site[b]			
1st tertile n=24			
Post-Commute	10.7	3.2	0.0014*
1 Hour after commute	16.2	3.8	<.0001*
2 Hours after commute	15.3	4.3	0.0007*
3 Hours after commute	14.8	4.8	0.0024*
2nd tertile n=28			
Post-Commute	10.1	3.3	0.0027*
1 Hour after commute	12.8	3.9	0.0016*
2 Hours after commute	14.2	4.4	0.0019*
3 Hours after commute	13.8	4.9	0.0056*
3rd tertile n=22			
Post-Commute	2.8	3.2	0.3848
1 Hour after commute	5.9	3.9	0.1384
2 Hours after commute	2.1	4.5	0.6393
3 Hours after commute	-2.8	5.0	0.5717

[a] SE=standard error

[b] tertiles increase in distance

* indicates significant at p-value<0.05

Table 3. Results of p-values from interaction models of individual characteristics with commute number and post-commute time points.

	Interaction Model				
Characteristic	With commute number[a]	With post-commute	With 1 hr post-commute	With 2 hrs post-commute	With 3 hrs post-commute
Sex	0.02*				
Healthy weight	0.6518	0.253	0.1315	0.1145	0.0534
Previous day's average ambient O_3	0.0098*				
Concurrent average ambient $PM_{2.5}$	0.0064*				
Distance to study site tertiles[b]		0.1904	0.1895	0.0826	0.0229*
1st tertile	0.1668	0.0997	0.072	0.0431*	0.0138*
2nd tertile	0.2032	0.1212	0.2187	0.0576	0.0177*
3rd tertile (ref)	-	-	-	-	-

[a] first commute or second commute

[b] tertiles increase in distance

* indicates significant p-value at $p<0.05$

Figure 1. Percent change in exhaled nitric oxide from baseline for all commutes.

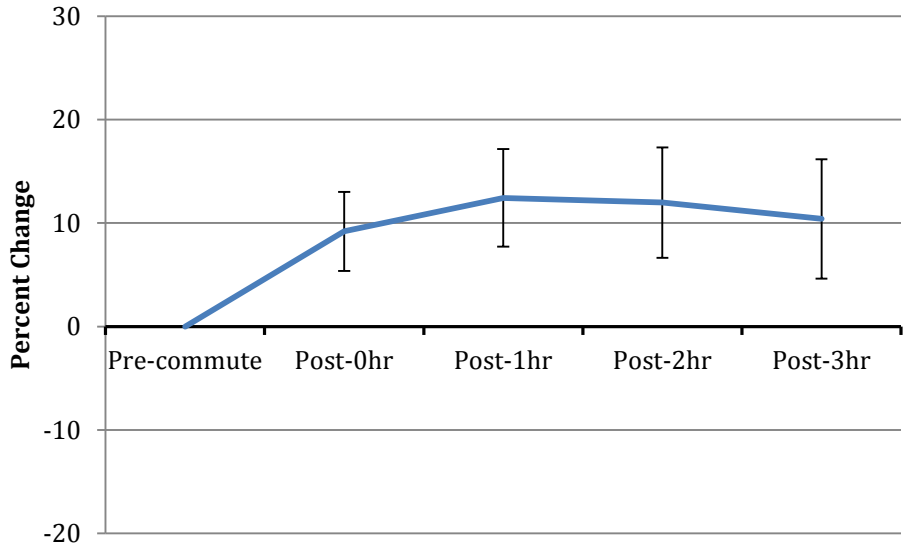


Figure 2. Percent change in exhaled nitric oxide from baseline by distance to study site tertiles.

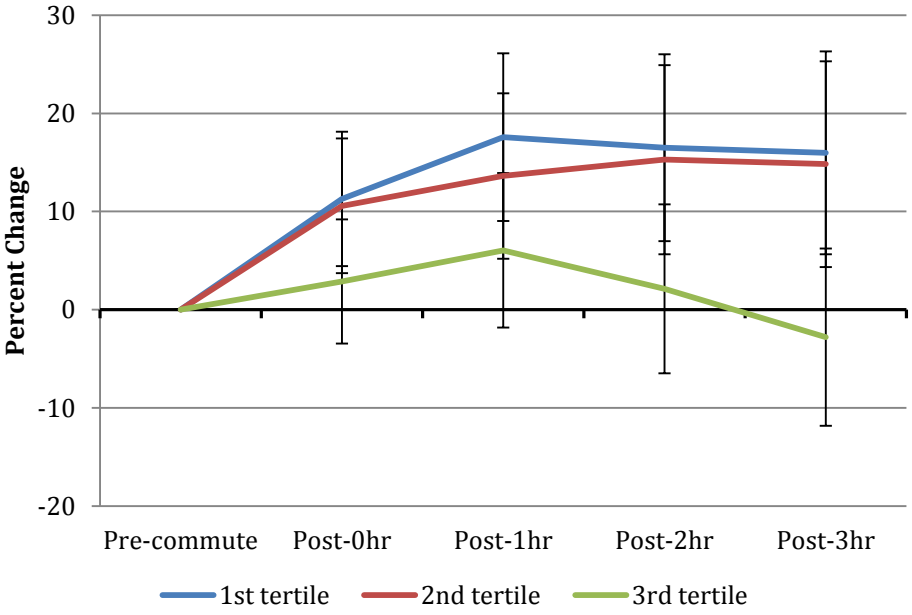
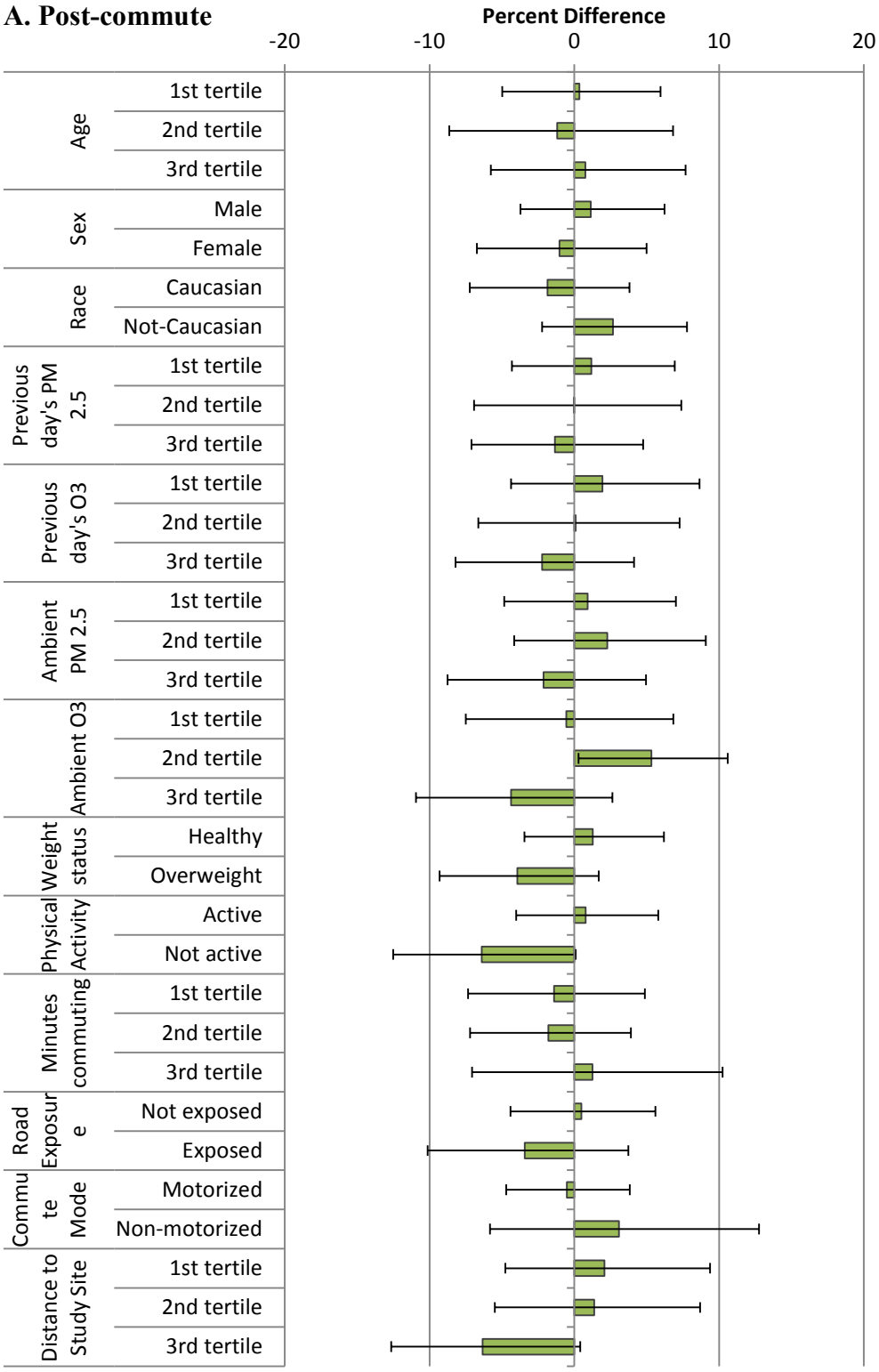
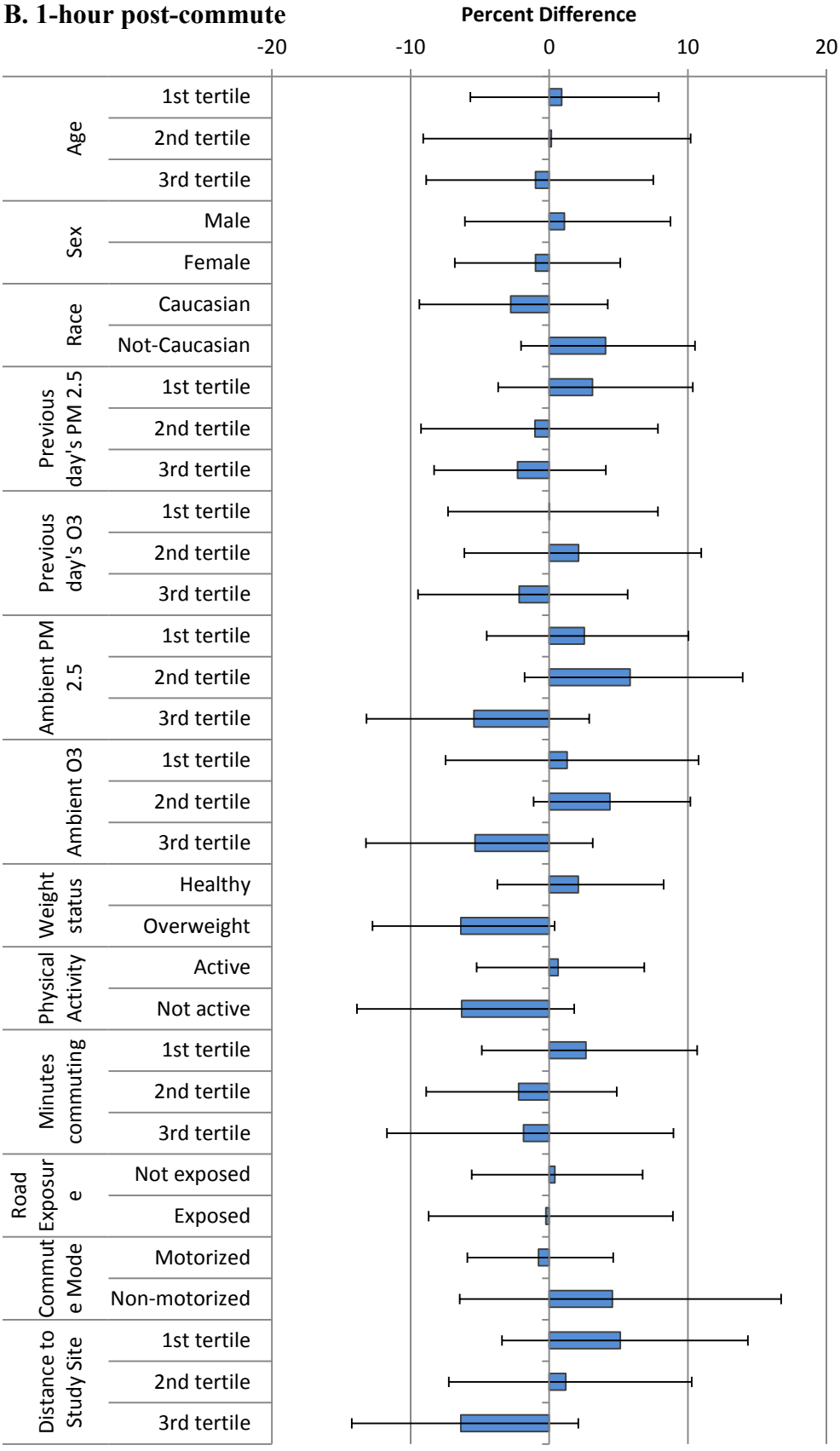


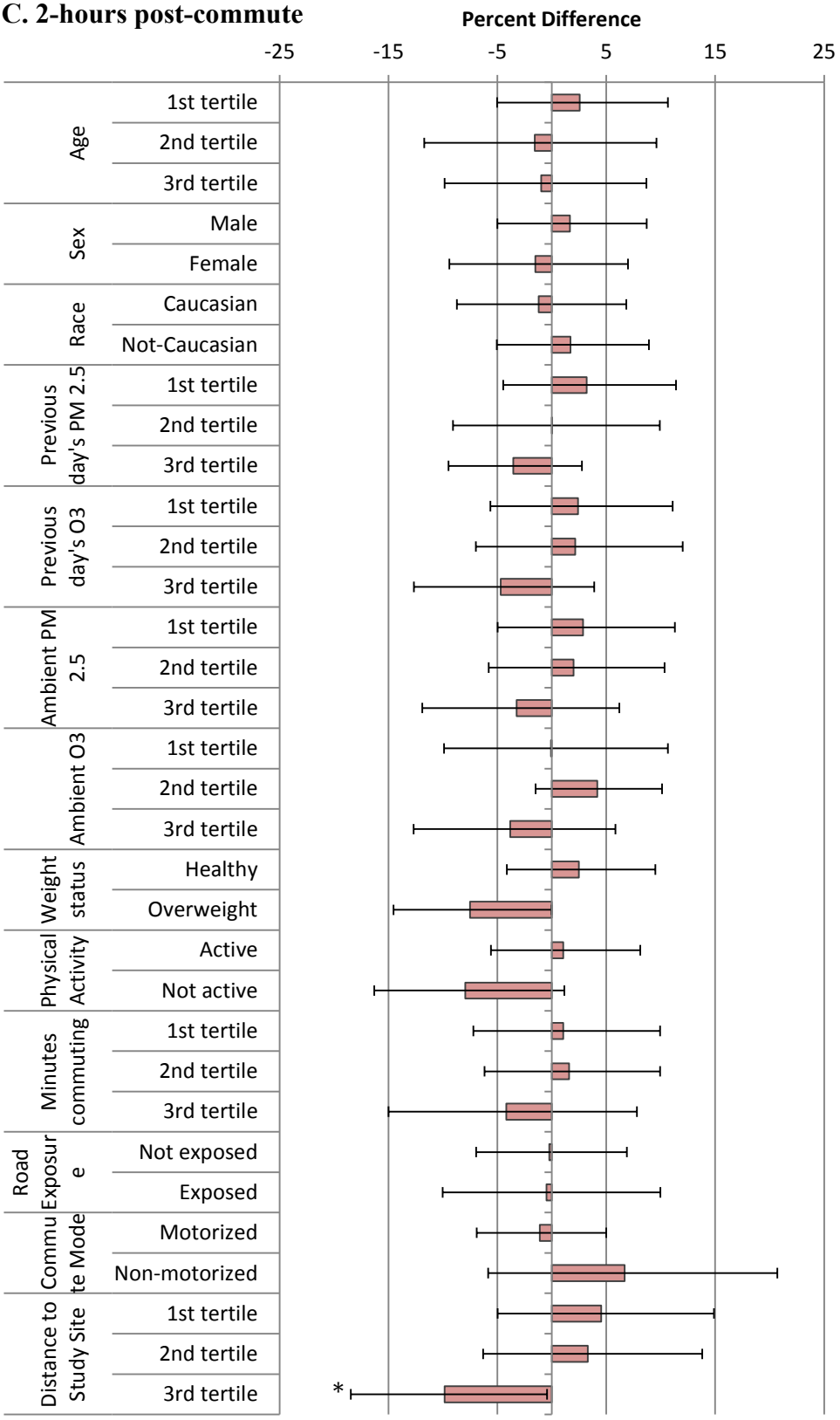
Figure 3. Percent change in eNO: difference between the percent change in eNO from the unstratified model and the corresponding percent change in the stratified models A. immediately post-commute; B. 1-hour after the commute; C. 2-hours after the commute; D. 3-hours after the commute.



B. 1-hour post-commute

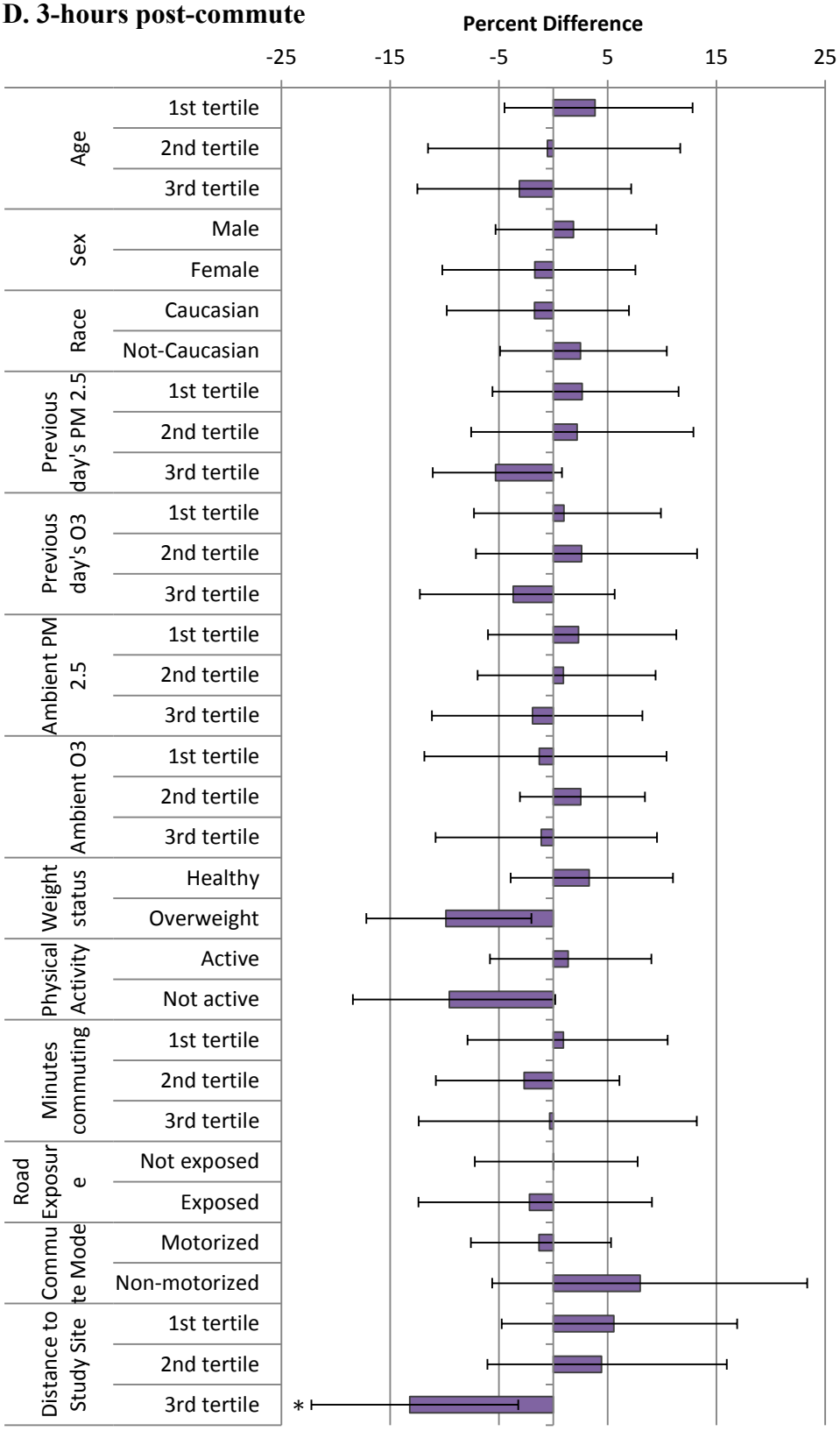


C. 2-hours post-commute



* significant from groups within the category at $p<0.05$

D. 3-hours post-commute



* significant from groups within the category at $p<0.05$