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Biological Markers of Asthma and Roadway Proximity

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Bachelor of Science
Emory University
2010

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An abstract of
A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
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Abstract

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Asthma is the most common chronic pediatric disease worldwide. Exposure to traffic-related air pollutants has been associated with adverse respiratory symptoms and several studies have shown that living near major roadways increases qualitative markers of asthma severity. My thesis seeks to 1) determine whether actual residential distance to a major roadway is associated with quantitative measures such as increased airway or systemic oxidative stress in asthmatic children and 2) determine whether residential distance to a major roadway and markers of oxidative stress are associated with clinical features of asthma severity in children. These questions were addressed through ArcGIS, a mapping tool used to measure residential distance from a major roadway, and database analysis of clinical and biological markers of asthma severity. Overall, patients who lived less than 300 meters from a major roadway experienced increased markers of systemic oxidative stress as measured by plasma cysteine/cystine, increased clinical markers of asthma severity as measured by wheeze incidence and β-agonsist use, decreased lung function and greater healthcare utilization compared to patients living greater than 300 meters from a major roadway. This suggests that traffic related air pollution might be a significant contributor to increased asthma symptoms and severity.

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INTRODUCTION

Asthma is the most common chronic pediatric disease worldwide and, according to the World Health Organization **(WHO),** is responsible for a large proportion of hospitalizations among children who are younger than 15. [1] Atlanta, Georgia, was recently named one of the "top 10 asthma capitals" of the United States due to the high prevalence and increased severity of asthma in this area. [2, 3] Atlanta is also plagued by some of America's highest numbers of smog alert days as well as heavy amounts of traffic. [4] Past research has shown that living near a major roadway is positively associated with increased asthma-related hospitalizations. [5] A California study showed that those living near heavy vehicular traffic were three times more likely to have an asthma-related emergency room or hospitalization when compared to those who lived near low vehicular traffic areas. [6] In other studies, exposure to traffic-related air pollutants has been associated with adverse respiratory symptoms. [7] The high exposures to vehicle emissions and by products of those living near roadways may confer increased vulnerability to pollution-mediated effects.[8] These repeated exposures to vehicle emissions may aggravate existing asthmatic symptoms among children already diagnosed with asthma. [9]

Asthma in children is a complicated disease associated with chronic airway inflammation and ongoing symptoms, airway obstruction, airway inflammation and bronchial hyperresponsiveness. [10] In the airways of asthmatic patients, there is increased size and proliferation of the upper airway cells as well as bronchial obstruction and bronchoconstriction. [11] Bronchial obstruction is when the airway is physically blocked and bronchoconstriction is defined by the American Lung Association as the constriction of the smooth muscle in the airways. [12] Due to the wide range and variability of symptoms, asthma characterization is further divided based on severity of these symptoms into mild-to-moderate and severe asthmatics. Mild-to-moderate asthmatics exhibit ongoing symptoms of asthma that are reversible through treatment. In contrast, severe asthmatics are characterized by persistence of symptoms despite treatments. The Global Health Initiative for Asthma, sponsored by the World Health Organization (WHO) and the National Heart, Blood and Lung Institute (NHLBI), defines severe asthma in the following manner: "symptoms prior to treatment are continuous, and punctuated by frequent exacerbations or frequent nighttime symptoms; impairment of lung function is demonstrated by the forced expiratory volume in one second (FEV_1) of <60% predicted, or peak expiratory flow variability of >30%; or there is limitation of daily physical activities by asthma symptoms."[13] FEV_1 is a pulmonary function test that measures the forced expiratory volume in one second that a patient can exhale. To conduct this test, a patient breathes into a mouthpiece connected to a spirometer, a machine that measures the rate and amount of air expelled by the patient. [14] FEV_1 and other pulmonary function test results are used to diagnose a variety lung diseases including asthma.

Asthma's causality is not well known, however, there are several indicators used in the clinical setting to diagnose the condition. After a patient complains of difficulty breathing or chest tightness, they undergo a series of pulmonary function tests and radiological tests such as chest radiographs or a computed tomography scan. [10] Pulmonary function tests indicate the volume of air that can be inhaled and expelled which, if low, can indicate blockage of the airways or an inability of the airways to expand due to fibrosis of the lungs. [10] Radiological tests can also reveal lung tissue abnormalities that may be hindering air intake capacity. [10] Many clinicians also test for bronchial hyperresponsiveness, a condition were bronchial spasms are easily triggered. Using a methacholine or histamine challenge to promote bronchoconstrictions, clinicians can measure the responsiveness of the airways as well as its reversibility. [10]

Studies have shown that vehicle emissions exposure is associated with higher risks of adverse respiratory health effects when compared to respiratory health effects of ambient air pollution. [5] In addition, the rate of adverse respiratory health effects and residential proximity to a roadway are inversely correlated. [8] These findings are supported by observations of high roadside concentrations of key traffic-related pollutants with high respiratory impacts (e.g. nitrogen oxide, black smoke and particulate matter) that fall exponentially to background levels within approximately 150 meters. [15] Closer proximity has been associated with increased rates of clinical and biological markers of asthma such as increased wheezing [16], respiratory effects, atopy and more frequent use of the health care system. [17, 18] Holguin et al. found that for each 50, 75 and 200 meter buffer, exhaled nitric oxide increased and forced expiratory volume per one second **(FEV_1)** decreased indicating increased signs of respiratory effects. [5] In addition to measuring individual asthma indicators, some studies have found there is an increased prevalence of asthma among children living within 100 meters of a major road and that the prevalence increased more sharply at 75 meters. [8] Previous studies have used proximal distance to a major road a useful approximate exposure to traffic-related air pollutants in lieu of measured concentrations of ambient air pollution as this metric may reflect variability in traffic exposures across the population. [1, 8, 19] It is important to note, however, that not all studies have observed significant associations between residential roadway proximity and respiratory disease [15, 20, 21]. The lack of effect observed in these studies may be attributable to the use of poor exposure surrogates, including nonspecific measures of residence such as ZIP codes [15] and/or the use of subjective outcome measures, such as self-reporting of symptoms [21]. Indeed, studies of roadway proximity utilizing objective measures of asthma have been few and limited. [15]

Traffic-related air pollution is comprised of a composite mixture of nitrogen oxides, particulate matter, carbon monoxide, sulfur oxides and other volatile organic compounds which are all airway irritants. [19] Previous studies have demonstrated biological plausibility for the association between exposure to these irritant compounds and poor lung function. [22] Oxidative stress, which is an overabundance of reactive nitrogen and oxygen species that results in cell damage, has been hypothesized as a potential biological pathway by which air pollution mediates effect. [22] To date, the relationship between biological and environmental factors, such as traffic emissions, in children is poorly understood and the degree to which traffic-related air pollution contributes to oxidative stress in asthmatic children exposed is not known. $19.7 billion is spent each year on asthma-related care and since the role of air pollution in the etiology and persistence of asthma among children is ill defined, this is an important public health problem that warrants further investigation. [23]

The objective of the current analysis was to determine whether asthmatic children living nearer to major roadways experience higher levels of oxidative stress and ultimately increased biomarkers and clinical features of asthma compared to asthmatic children living further from major roadways. The study used for this thesis offers a new cohort of pediatric patients with mild/moderate or severe asthma with ample well-defined clinical data that is unique to air quality studies. In addition, this study uses objective clinical and biological markers of asthma severity and contains many unique measures to address biological impacts of air quality. Analysis was performed using samples collected from children previously enrolled and characterized according to criteria outlined by the National Heart, Lung and Blood Institute's Severe Asthma Research Program (SARP). The following specific aims were tested:

1. Determine whether residential distance to a major roadway is associated with increased oxidative stress in the airways or systemic circulation of asthmatic children.
2. Determine whether residential distance to a major roadway and markers of oxidative stress are associated with clinical features of asthma severity in children.

METHODS

This study was conducted using previously-collected and analyzed samples of plasma, bronchoalveolar lavage **(BAL)**, and exhaled breath condensate **(EBC)** biomarker data from children with mild-to-moderate and severe asthma enrolled in the National Institutes of Health/National Heart, Lung and Blood Institute's Severe Asthma Research Program **(SARP)**. SARP is an ongoing multicenter study that began with patient enrollment in 2001. In order to investigate the clinical and biological attributes of severe versus mild-to-moderate asthma, SARP has developed a definition of severe asthma along with a Manual of Procedures, which includes standard questionnaires and procedures for pulmonary function testing, allergy testing, and bronchoprovocation. Emory University has served as the sole pediatric recruiting site for SARP. To date, 211 asthmatic children, aged 5-17, have been recruited at Emory University and have provided plasma, exhaled breath condensate **(EBC)**, and bronchoalveolar lavage **(BAL)** samples. These samples are housed in the Emory University Department of Pediatrics and are under the discretion of Dr. Anne Fitzpatrick (Emory SARP PI). Data for 195 SARP participants were available for the current analysis.

Subject characterization and Health Outcome Data

Children enrolled in SARP previously underwent detailed characterization consisting of medical history and symptom questionnaires, spirometry, plethysmography, allergy evaluation, exhaled nitric oxide sampling, and methacholine challenge. These characterization procedures were described previously. [24] Briefly, spirometry was performed at baseline and 15 minutes after receiving 2 inhalations of albuterol sulfate (90 μg/inhalation). The results fulfilled criteria for reproducibility and were interpreted according to reference standards.[25] Lung volumes were measured with a body plethysmograph and expressed according to reference standards.[26] Broncho-provocation testing was limited to children with a baseline FEV_1 > or < 70% predicted and was performed using 10 concentrations of methacholine from 0 to 25 mg/mL delivered by a Rosenthal dosimeter.[27] Allergy skin prick testing was performed with a standard kit (Multi-Test II) containing tree pollen, grass pollen, ragweed pollen, weed pollen, dog hair, cat epithelium, alternaria, cladosporidium, aspergillus, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, and cockroach extracts. Exhaled nitric oxide was collected with a reservoir bag at a fixed exhaled flow rate of 0.35 L/s.[28]

For the current analysis using the existing database built by SARP, systemic oxidative stress was assessed as follows: 1) plasma concentrations of isoprostanes, a lipid peroxidation product; 2) plasma concentrations of glutathione, an antioxidant; 3) cysteine, a sulfur amino acid that is essential for glutathione synthesis, and 4) plasma concentrations of 8-hydroxy-2'-deoxyguanosine, a by-product of DNA oxidation. [29-32] Pulmonary oxidative stress was assessed using exhaled total nitric oxide concentrations. Clinical features of asthma severity, as used in other studies, included wheezing at least twice a week, using a β-agonist at least once a day, having been hospitalized in the last year, airway resistance as measured by the sRAW percent predicted baseline, and lung function as measured by FEV_1/FVC percent predicted baseline and maximum ratios.[33]

Proximity to Roadway Data

Exposure to traffic pollution was assessed using the patient's residential distance from a major roadway which was calculated using geocoding and GIS measuring techniques available through ArcGIS. Patient addresses were geocoded using the ArcGIS geocoder. Those that were not matched (n=27) by ArcGIS were matched using GoogleEarth to obtain X and Y coordinates that were then entered into ArcGIS. A subset of ten address records that were geocoded using ArcGIS were then geocoded using GoogleEarth to validate that identical results were obtained from ArcGIS and GoogleEarth. The map of the patient's addresses was then overlaid with the major roadways of Georgia. The major roadways were obtained from the 2010 National Transportation Atlas Database from The Bureau of Transportation Statistics (BTS). [34] This analysis considered major roadways to be all state highways and interstates as dictated by the United States Department of Transportation. Using the ArcGIS measuring tool, the distance between each subject's home and the closest major roadway [e.g. distances between all of the points (addresses) to the nearest line segment (roadways)] was calculated in meters **(Image 1)**. Residential distance from a major roadway was divided into those living less than 300 meters from a major roadway and patients living at least 300 meters or greater from a major roadway. This division was based on past research demonstrating that the majority of lung function effects are observed at the 300 meter division [5] and the concentration of emitted motor vehicle pollutants is highest within 150 meters of a roadway and dramatically decreases after 300 meters. [5, 35, 36].

Epidemiological Analysis

The association between residential distance from a major roadway and measures of asthma severity were assessed using logistic regression, correlation and analysis of variance (ANOVA) techniques in PASW (formerly SPSS). Potential confounders and covariates of this relationship, such as race and socioeconomic status (SES, as determined by highest level of parental education), were also examined. More specifically, logistic regression was used to assess the relationship between residential proximity to a major roadway and dichotomous outcome measures including highest parental education, wheezing at least twice weekly, daily β-agonist use, and hospitalizations in the last year. The ANOVA technique was used to assess the relationship between residential proximity to a major roadway and continuous outcome measures, including air resistance (sRAW), FEV_1/FVC baseline percent predicted, and the maximum FEV_1/FVC percent predicted. Correlation techniques were used to assess the relationship between residential proximity to a major roadway as a continuous exposure measure and the continuous outcome variables. No variables required logarithmic transformation for this analysis.

RESULTS

Study Population Demographics

The features of the 195 participating children are summarized in Table 1. The average age was 11 ± 4 years. Fifty-five percent of the combined sample were male and 57% were African American. In terms of asthma severity, 54% percent of participating children had severe asthma while 46% had mild-to-moderate asthma. Age and sex did not differ between children with severe versus mild-to-moderate asthma. However, there were significantly ($p < 0.05$) more African Americans with severe asthma (72%) compared to Caucasians with severe asthma (40%). Severe asthmatics were also significantly younger at the age of diagnosis (2 ± 4 vs. 4 ± 4 years, $p < 0.05$), and more likely to have made a visit to the ER in their lifetime (93% vs. 53%, $p < 0.05$), and were almost six times more likely to have been hospitalized when compared to mild-to-moderate asthmatics ($p < 0.05$). Per the SARP definition, lung function as measured by baseline percent predicted FEV_1 was also significantly lower in those with severe asthma (83 ± 19 vs. 97 ± 16%, $p < 0.01$).

The features of participating children according to residential proximity to a major roadway are also shown in **Table 1**. Twenty-three children lived less than 300 meters and 172 children lived at least 300 meters from a major roadway. There were no statistical differences in the measured subject characteristics (age, race, age of diagnosis, severe asthma, inhaled corticosteroid use, IgE score, pet dander trigger, positive allergy skin prick test) between patients who lived less than 300 meters from a major roadway when compared to those who lived greater than 300 meters from a major roadway.

Notably, the percent of severe asthmatics was not significantly different among those living less than 300 meters (62.5%) and those living at least 300 meters from a major roadway (52.3%), although a slight trend was observed. Additionally, there was no difference in SES, as measured by parental education level, between patients who lived less than 300 meters from a major roadway compared to those who lived greater than 300 meters from a major roadway.

Relationship to Markers of Oxidative Stress

To determine the relationship between residential proximity to a major roadway and markers of oxidative stress, correlation analyses were performed using proximity to a major roadway as a continuous variable. The measures of oxidative stress used were plasma isoprostane, plasma glutathione, plasma cysteine concentration, cysteine/cystine redox potential, deacetylase, 8-hydroxy-2'-deoxyguanosine (8-OhdG), and exhaled nitric oxide **(Table 2)**. The relationship between residential roadway proximity and plasma isoprostane was not significant. The relationship between residential roadway proximity and plasma glutathione was also not significant but the correlations with roadway proximity were in the same direction as the redox potential which demonstrates consistency between the associations. While the relationship between residential roadway proximity and plasma cysteine concentration was not significant, the correlation between residential roadway proximity and the percent of cysteine/cystine redox potential was significant **(Figure 1)**. When measuring redox potential, the more negative the redox potential the greater the potential for cysteine to detoxify an injurious radical, making for a healthier lung.[37] A more positive redox potential indicates that the lung tissue has been more heavily oxidized and indicates more oxidative stress and worse lung health [38]. There was no significant correlation between residential proximity to a major roadway and 8-OhdG or with exhaled nitric oxide.

Relationship to Clinical Asthma Features

Relationship to Persistence of Clinical Asthma Symptoms

Clinical asthma symptoms were measured by six clinical markers that were chosen a priori from roughly fifty available clinical markers of asthma based on questionnaire and clinic visit patient responses **(Table 3)**. To determine the relationship between residential proximity to a major roadway and clinical features of asthma severity in children, logistic regression analyses were performed using proximity to a major roadway as a dichotomous variable (< 300 meters vs. > 300 meters). Most associations showed trends in the expected direction, with significant associations observed between residential proximity to a major roadway and daily β-agonist use and wheezing at least two times a week. β-agonists are bronchodilator medications that relax the airway muscles in order to widen the airway making breathing easier [39]. We found that the farther away patients lived from a major roadway, the less likely patients were to have used daily β-agonists (OR: 0.408, CI 0.167, 0.301) **(Figure 2).** Among patients who lived 300 meters or less from a major roadway, 61% reported daily β-agonists use compared to only 38% of those living greater than 300 meters from a major roadway (p-value 0.046). Increased residential distance to a major roadway was also significantly negatively associated with wheezing at least two times a week (OR: 0.351, CI 0.145, 0.852) **(Figure 3).** Among patients living 300 meters or less from a major roadway, 52% reported wheezing at least twice a week compared to 27% among those who lived greater than 300 meters from a major roadway (OR:0.351, CI 0.145, 0.852).

Relationship To Lung Function

In this sample, closer residential proximity to a major roadway was significantly associated with decreased lung function as measured by specific airway resistance **(sRaw)** percent predicted and the forced expiratory volume in one second **(FEV_1)** to forced vital capacity **(FVC)**. The sRaw percent predicted was measured via plethysmography using changes in airflow and changes in plethysmographic pressure and results in the product of functional residual capacity **(FRC)** and airways resistance.[40] The greater the sRaw score, the greater the airway resistance. Patients with a residence close to a major roadway experienced greater airway resistance than those living further away from a major roadway **(Figure 4)**. FEV_1 is used as an indicator of asthma severity because it gauges lung capacity and the lower the FEV_1 the more severe the asthma diagnosis.[41] FVC is the total volume of air that can be forcibly exhaled given a maximal inspiration.[42] The FEV_1/FVC ratio is the calculated proportion of the forced vital capacity exhaled in one second and is based on the percent predicted value before and after bronchodilation.[43] A higher percent predicted value is, therefore, indicative of better lung function. Patients in this cohort who lived greater than 300 meters from a major roadway experienced higher FEV_1/FVC scores before **(Figure 5)** and after **(Figure 6)** bronchodilation which indicated better lung function compared to those who lived 300 meters or less from a major roadway.

Relationship to Healthcare Utilization

Patients living 300 meters or less from a major roadway had significantly more healthcare utilization as measured by the number of hospitalizations in the previous year compared to patients living greater than 300 meters from a major roadway **(Figure 7)**. Among patients who lived less than 300 meters from a major roadway, 57% had been hospitalized in twelve months prior to their clinic visit and completion of the questionnaire while only 35% of those living 300 meters or more from a major roadway had been hospitalized during the same time frame.

DISCUSSION

Asthma is highly prevalent yet complex disease that epidemiological studies have shown has two necessary components in order to develop: 1) genetic factors and 2) environmental factors.[44] Studies have shown that environments with pet dander, microbes, pollen and air pollution promote asthma development.[44] Furthermore, numerous studies have demonstrated that proximity to roadways and consequently roadway traffic exhaust as measured by physical distance, roadway density and traffic density, has been associated with increased asthma symptoms in children.[1, 5-8, 45] However, these previous studies were based on evaluations of asthma by either the child or by the parent, which may have introduced subjectivity and noise to the outcome measures. What has not been previously determined is the use of objective measures of lung function and severity to measure the relationship between roadway proximity and asthma. The current study is unique in that it employed both subjective and objective characterization data from the SARP database to evaluate the impacts of residential roadway proximity on asthma outomces.

This study supports the hypothesis that farther residential distance from a major roadway provides a protective effect from asthma symptoms of severity and is consistent with other reports.[5, 8] This study found that children with asthma who live 300 meters or less from a major roadway experience greater clinical and biological markers of asthma. There was an association between living 300 meters or less from a major roadway and increased clinical symptoms of asthma as measured by an increased prevalence in daily β-agonists use, wheezing at least twice a week, and hospitalizations in the previous twelve months. There was also an association between living less than 300 meters from a major roadway and biological markers of asthma for lung function as measured by airway resistance and the FEV_1/FVC percent predicted before and after bronchodilation. Importantly, there was no statistical difference between residential proximity to a major roadway between severe and mild-to-moderate asthmatics which demonstrates that the analysis was not confounded by more severe asthmatics living closer to major roadways. Moreover, there was no association between residential roadway proximity and SES indicating that the analysis was likely not confounded by SES differences.

β-agonists are used to relieve asthma symptoms of chest tightness and air constriction; thereby reflecting how often a patient is experiencing asthma symptoms and, consequently, asthma severity. Although the prevalence of SARP-defined severe asthma was not different according to major roadway proximity, this study found that of patients who lived less than 300 meters from a major roadway, roughly a third more used β-agonists on a daily basis than patients who lived 300 meters or more from a major roadway. Additionally, almost twice the number of patients who lived less than 300 meters from a major roadway experienced wheezing at least two times a week compared to patients who lived greater than 300 meters from a major roadway. Thus, patients living nearer to a major roadway exhibited a greater number of asthma symptoms and therefore severity despite better medication controls as measured by β-agonists use. This indicates that patients living closer to a major roadway are significantly more impacted by asthma symptoms of chest tightness and air way constriction when compared to those who live farther from a major roadway.

Measures of lung function were used as one of the objective measures of severity in this study. This study found that patients living less than 300 meters from a major roadway had significantly lower lung function compared to patients who lived greater than 300 meters from a major roadway. Specifically, they experienced significantly more airway resistance which indicates how well air is moving in and out of a patient's lungs which means that there are increased mechanical factors limiting inspired air to reach the pulmonary alveoli and result in less air flow. This same group of patients also had decreased lung function as measured by the baseline FEV_1/FVC percent predicted ratio and the maximum FEV_1/FVC ratio. Lower FEV_1/FVC percent predicted ratio scores indicated that patients living less than 300 meters from a major roadway had a lower lung capacity, meaning their lungs are less capable of expanding and contracting to full capacity, when compared to patients living at least 300 meters from a major roadway. Patients living less than 300 meters from a major roadway not only had decreased lung capacity but they also experienced less reversibility meaning that even after treatment with medication that should promote bronchodilation, they showed less improvement compared to children who live at least 300 meters from a major roadway who also received bronchodilators. It is important to note that patients living less than 300 meters from a major roadway exhibited decreased lung function despite increased β-agonists use.

Patients living closer than 300 meters from a major roadway had greater healthcare utilization compared to those who lived at 300 meters from a major roadway with slightly more than one and half times greater hospitalizations in the previous year. The patients experienced greater healthcare utilization despite increased β-agonists use when compared to patients who lived greater than 300 meters from a major roadway. These patients also exhibited more severe symptoms as their lung function is was compromised that they not only went to the emergency room but needed to be hospitalized, the latter of which was captured by this study. This suggests that of the $19 billion a year that is spent on asthma related costs, patients living closer to major roadways are disproportionately represented in this cost.[23] Specifically, asthma affects one out of every ten children in Georgia and they experience numerous direct and indirect costs as a consequence.[2] These include direct costs of medical care and prescription drugs as well as indirect costs of roughly $5.1 billion from activity limitation, death and missed school days.[23] Missed school days due to asthma complications is a major problem given that there are roughly 12.8 million missed school days among asthma patients nationwide.[44] Given that patients enrolled in this study are under eighteen and had an average age of 11, missing school puts them at a severe disadvantage in their education.

The significant correlation between residential distance from a major roadway and the redox potential of plasma cysteine/cystine reflects that there was significantly more oxidative stress among patients living nearer to major roadways despite no significant difference in severity. Plasma cysteine/cystine is a marker of systemic oxidative stress. Observation of a significant association between roadway proximity and oxidative stress measured in plasma, and not merely exhaled breath condensate, suggests that the reactive oxygen species were numerous enough to leave and subsist outside of the alveolar space

[37] and that there was substantial reactive oxygen species burden in those asthmatic children living less than 300 meters from a major roadway. This general increase in oxidative stress may provide mechanism by which subjects living within 300 meters also had increased asthma symptoms.

The results of this study may have been limited by lacking a traffic density factor but secondarily controlled for by the Department of Transportation definition of a major highway or interstate which by design is a high traffic volume roadway. The results of this study might also have been limited by the use of some questionnaire results as subjective measures of asthma severity rather than all objective measures. However, objective measures were also presented in this study. Lifetime history at the patient's residence was not available for analysis and may have contributed to some exposure measurement error. This study was also limited by the lack of directly measured air pollutant concentrations near each patients residence. This analysis was also limited by the lack of power to conduct multiple regression analysis to directly control for potential confounders such as SES.

In summary, we have shown that children who lived less than 300 meters from a major roadway had increased oxidative stress and symptoms of asthma severity regardless of their asthma severity classification of mild-to-moderate or severe. Patients who lived less than 300 meters from a major roadway had increased asthma symptoms, decreased lung function and increased healthcare utilization despite increased β-agonists use. This suggests that traffic related air pollution might be a significant contributor to increased asthma symptoms and severity. Future studies might include additional objective markers of asthma severity and traffic density measures where available. Ambient air readings near roadways might also further help to understand the relationship between pediatric asthma and traffic related air pollution by delving into the underlying pollutant components and the mechanisms involved.

APPENDIX

Table 1. Study Population Demographics by Roadway Proximity

		Total Sample n= 195	**<300 Meters from Major Roadway n= 23**	**>300 Meters from Major Roadway n= 172**
Age (in years)		11.64 ± 3.45	10.89 ± 2.90	11.74 ± 3.51
Males		54.9%	52.2%	55.2%
African American		**56.9%**	52.2%	57.6%
Age when diagnosed		3.21 ± 4.02	3.04 ± 3.15	3.24 ± 4.13
% severe asthma (SARP Criteria)		53.8%	62.5%	52.3%
% taking ICS		89.2%	88.4%	89.2%
Log serum IgE (kU/L)		5.14 ± 1.75	5.36 ± 1.80	5.11 ± 1.75
% with pet trigger		40.1%	43.5%	39.6%
Positive allergy skin prick test		2.85 ± 2.77	3.22 ± 2.39	2.79 ± 2.83
Education level	**Did not complete high school**	8.4%	9.1%	8.3%
	High school degree	14.0%	18.2%	13.5%
	Some college or more	77.7%	72.7%	78.1%

Data are shown as the mean ± standard deviation or the frequency percentage.

Table 2. Pearson correlations between residential roadway proximity as a continuous measure and Biological Markers of Oxidative Stress

		Distance in Meters
Plasma isoprostane	**Pearson Correlation**	-0.251
	N	36*
Plasma cysteine/cystine	**Pearson Correlation**	**-0.377**
	N	30*
Plasma glutathione	**Pearson Correlation**	-0.234
	N	30*
8-OhdG	**Pearson Correlation**	0.082
	N	65
Log 8-OhdG	**Pearson Correlation**	0.101
	N	65
Online exhaled nitric oxide	**Pearson Correlation**	-0.119
	N	96
Log online exhaled nitric oxide	**Pearson Correlation**	-0.121
	N	96

*Biomarker data was only available for a subset of participants

Table 3. Clinical Markers of Asthma Severity by Roadway Proximity

	Total Sample n= 195	<300 Meters from Major Roadway n= 23	>300 Meters from Major Roadway n= 172
Daily β-agonist use	42.4%	62%	38%
Wheezes ≥ 2x a week	31.6%	52%	27%
sRaw	131.5 ± 66.4	164.9 ± 104.8	124.95 ± 54.5
FEV1/FVC baseline % predicted	89.4 ± 12.3	85.5 ± 10.9	91.9 ± 12.3
Maximum FEV1/FVC % predicted	95.01 ± 10.04	93.3 ± 8.9	97.1 ± 10.2
Hospitalized in previous 12 months	40.6%	57%	35%

Image and Figure Legends

Image 1.

Patients residential addresses mapped with an overlay of major roadways in Georgia. The majority of patients live within the metro Atlanta area with a few patients living in rural counties. There was no statistical difference in severity as measured by SARP criteria between patients living less than 300 meters from a major roadway and those living greater than 300 meters from a major roadway.

Figure 1.

More positive cysteine/cystine redox potential correlates with roadway proximity in this sample. A more positive cysteine/cystine redox potential is indicative of a more oxidized airspace which equates to more oxidative stress and worse lung function. Patients living less than 300 meters from a major roadway experienced more positive redox potential as measured by cysteine/cystine compared to those who lived at least 300 meters from a major roadway.

Figure 2.

Increased daily β-agonists use correlates with roadway proximity in this sample. Daily β-agonists use was higher in patients who lived less than 300 meters from a major roadway with a p-value of 0.049 and a 95% confidence interval from 0.167 to 0.996. In this sample 60.87% of patients living less than 300 meters from a major roadway used β-agonists medication daily compared to 37.21% of patients who lived at least 300 meters from a major roadway and experienced daily β-agonists use.

Figure 3.

Wheezing at least twice a week correlates with roadway proximity in this sample. Wheezing at least twice a week was higher in patients who lived less than 300 meters from a major roadway with a p-value of 0.021 and a 95% confidence interval from 0.145 to 0.852. In this sample, 52% of patients living less than 300 meters from a major roadway wheezed at least twice a week compared to 27% of patients who lived at least 300 meters from a major roadway that wheezed at least twice a week.

Figure 4.

Greater airway resistance correlates with roadway proximity in this sample. Airway resistance was higher in patients who lived less than 300 meters from a major roadway with a p-value of 0.012 and a r-squared value of 0.05. In patients who lived less than 300 meters from a major roadway the median airway resistance value 164.9 with a 25th percentile value of 101.0 and a 75th percentile value of 180.3. In patients who lived at least 300 meters from a major roadway the median airway resistance value 124.95 with a 25th percentile value of 90.5 and a 75th percentile value of 151.7.

Figure 5.

FEV_1/FVC percent predicted ratio correlates with roadway proximity in this sample. The FEV1/FVC percent predicted ratio was lower in patients who lived less than 300 meters from a major roadway with a p-value of 0.062 and a r-squared value of 0.018. In patients who lived less than 300 meters from a major roadway, the median value was 85.5 with a 25th percentile value of 78.4 and a 75th percentile value of 93.8. In patients who lived at least 300 meters from a major roadway, the median value was 91.9 with a 25th percentile value of 82.2 and a 75th percentile value of 97.8.

Figure 6.

Maximum FEV_1/FVC ratio after bronchodilation correlates with roadway proximity in this sample. The maximum FEV_1/FVC ratio after bronchodilation was lower in patients who lived less than 300 meters from a major roadway with a p-value of .039 and a r-squared value of 0.023. In patients who lived less than 300 meters from a major roadway, the median value was 93.3 with a 25th percentile value of 83.4 and a 75th percentile value of 98.1. In patients who lived at least 300 meters from a major roadway, the median value was 97.1 with a 25th percentile value of 90.9 and a 75th percentile value of 103.

Figure 7.

Hospitalization in the last year correlates with roadway proximity in this sample. Hospitalizations was higher in patients who lived less than 300 meters from a major roadway with a p-value of 0.051 and a 95% confidence interval from 0.172 to 1.005. In this sample 52% of patients living less than 300 meters from a major roadway had been hospitalized in the previous twelve months compared to 34% of patients who lived at least 300 meters from a major roadway that had been hospitalized in the previous twelve months.

Image 1.

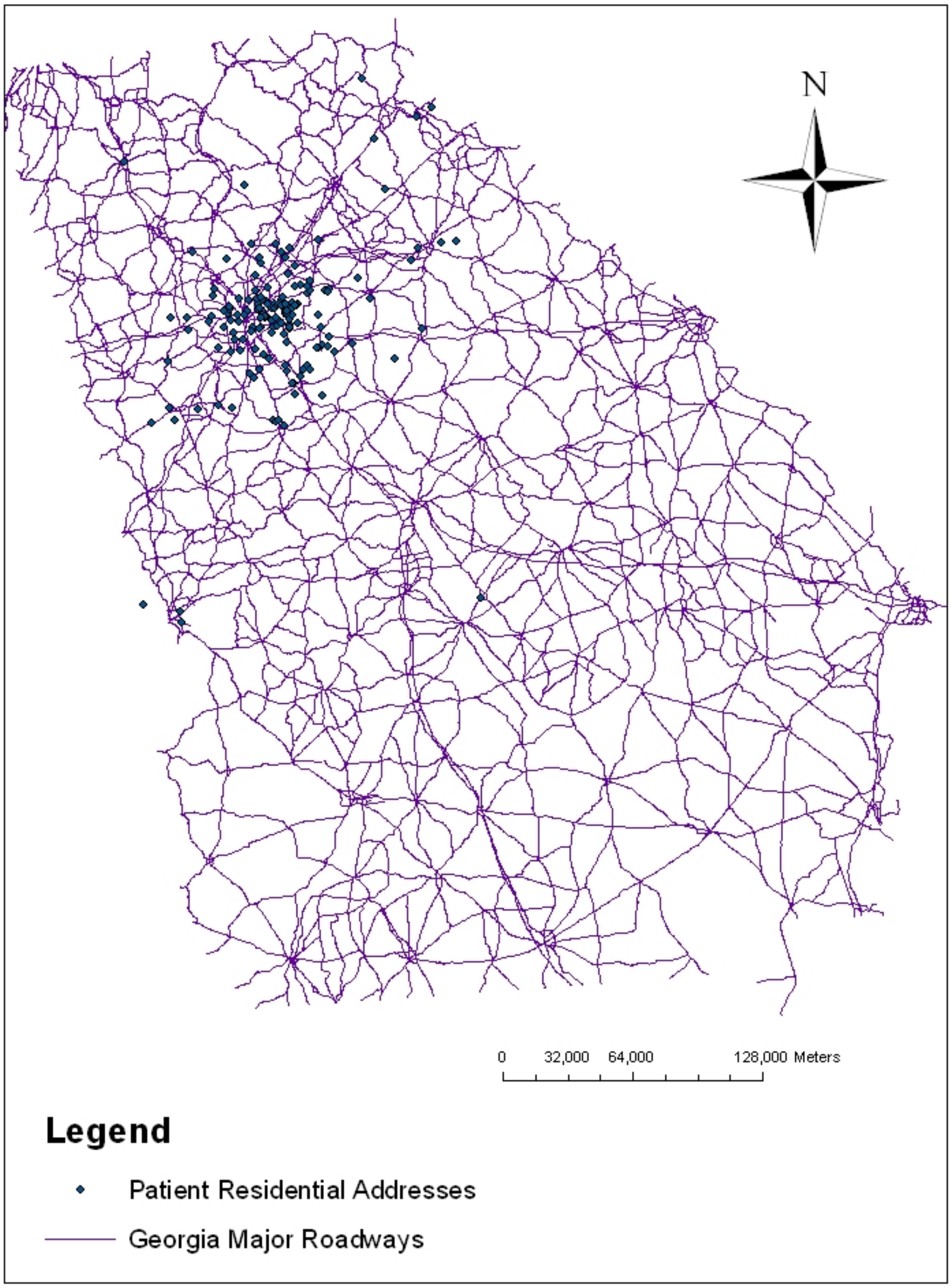

N
0
32,000
64,000
128,000 Meters
Legend
◆ Patient Residential Addresses
Georgia Major Roadways

Figure 1.

Cysteine/Cystine Redox Potential

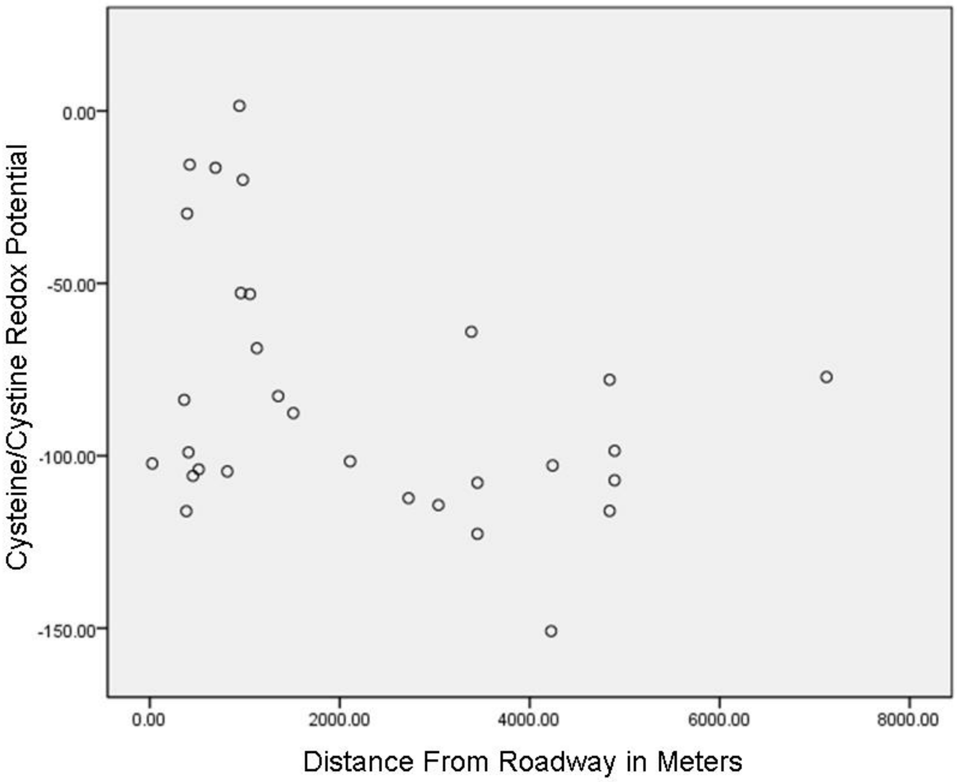


Figure 2.

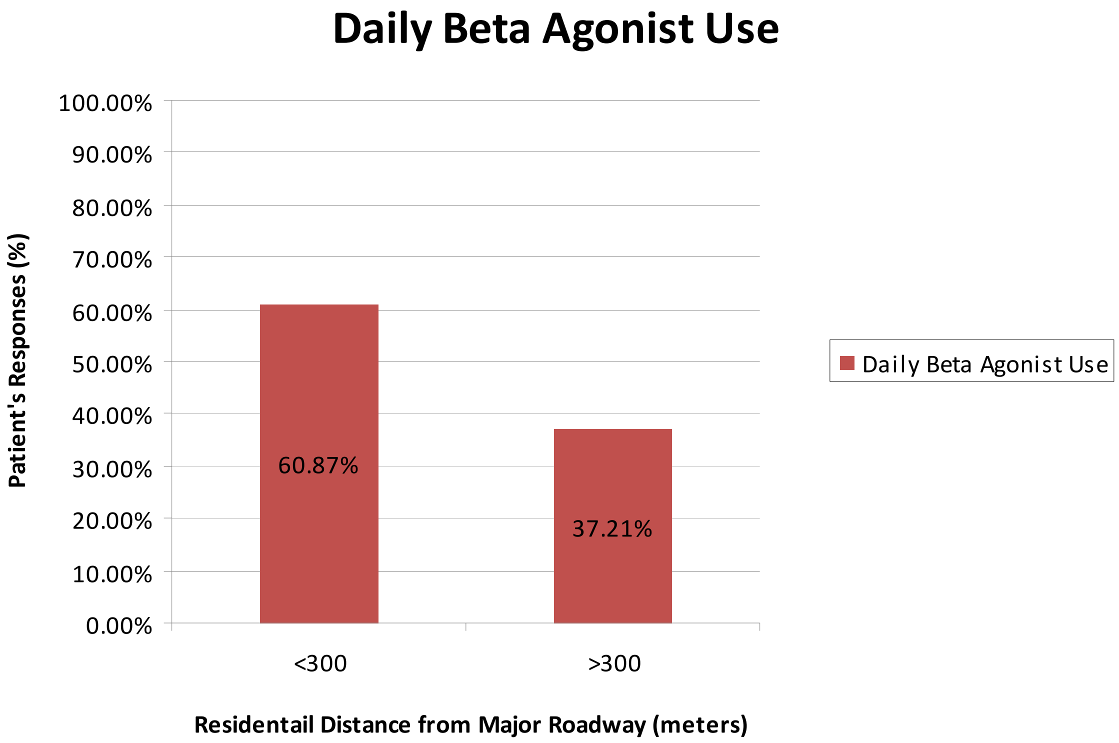

Daily Beta Agonist Use
100.00%
90.00%
80.00%
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
Patient's Responses (%)
60.87%
37.21%
<300
>300
Residentail Distance from Major Roadway (meters)
Daily Beta Agonist Use

Figure 3.

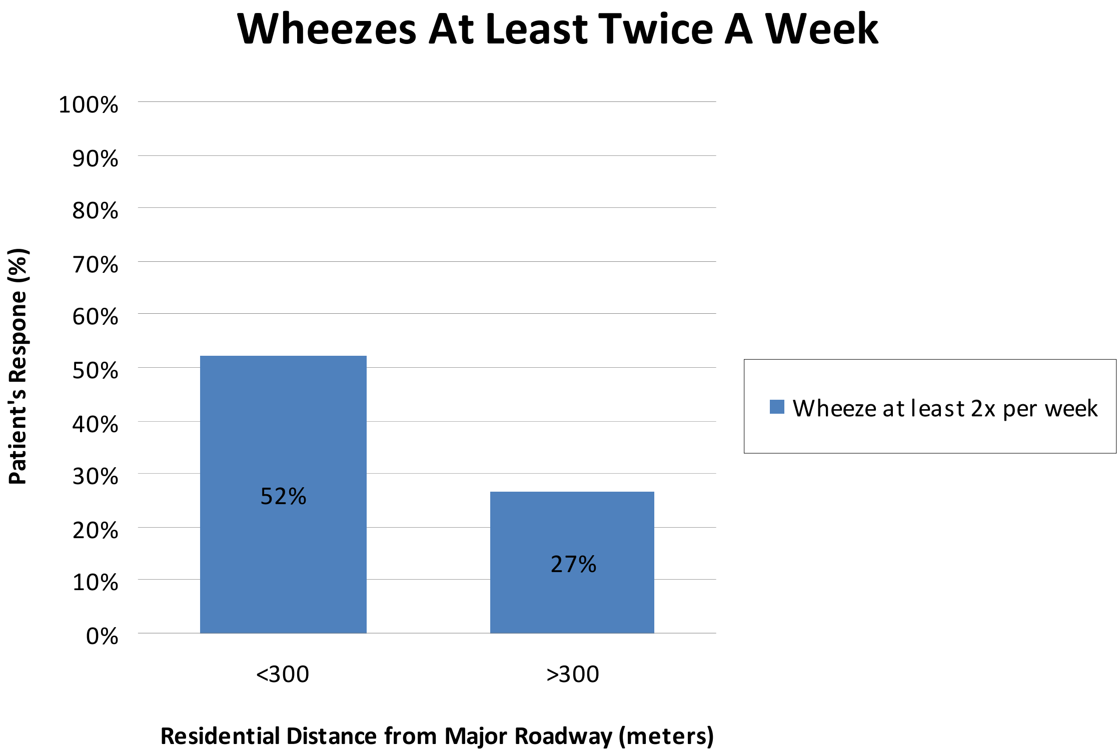

Wheezes At Least Twice A Week
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Patient's Respone (%)
52%
27%
<300
>300
Residential Distance from Major Roadway (meters)
Wheeze at least 2x per week

Figure 4.

Airway Resistance

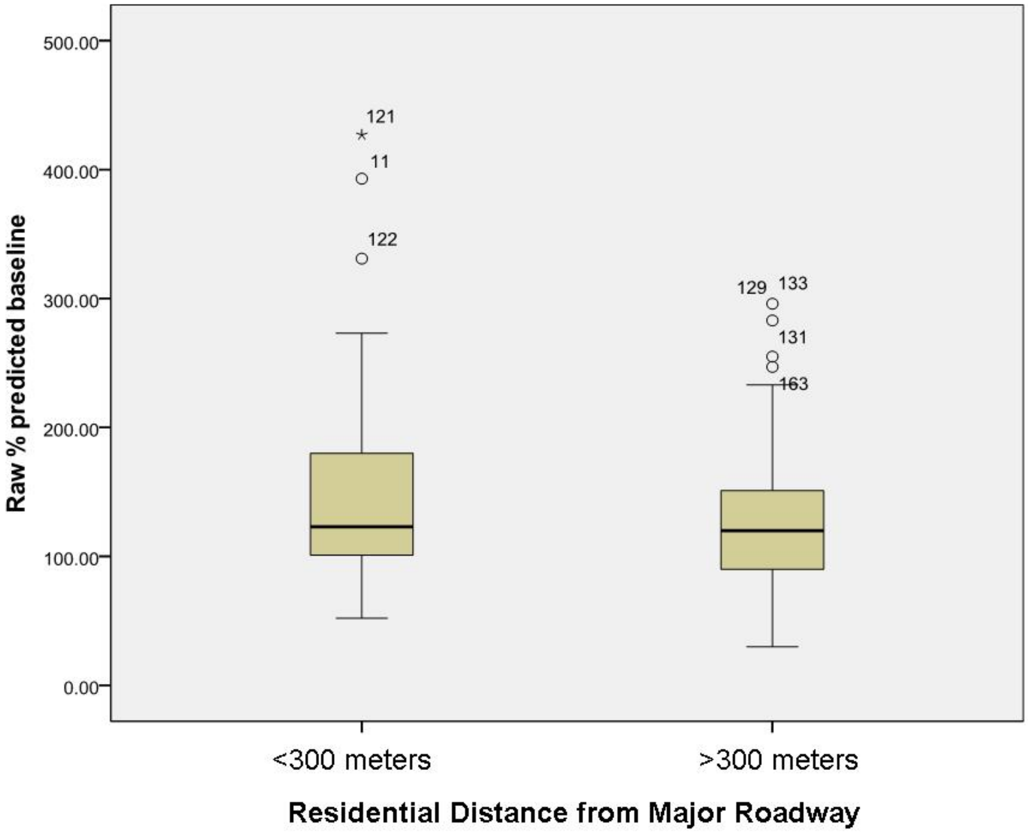


Figure 5.

FEV_1/FVC % Predicted Baseline

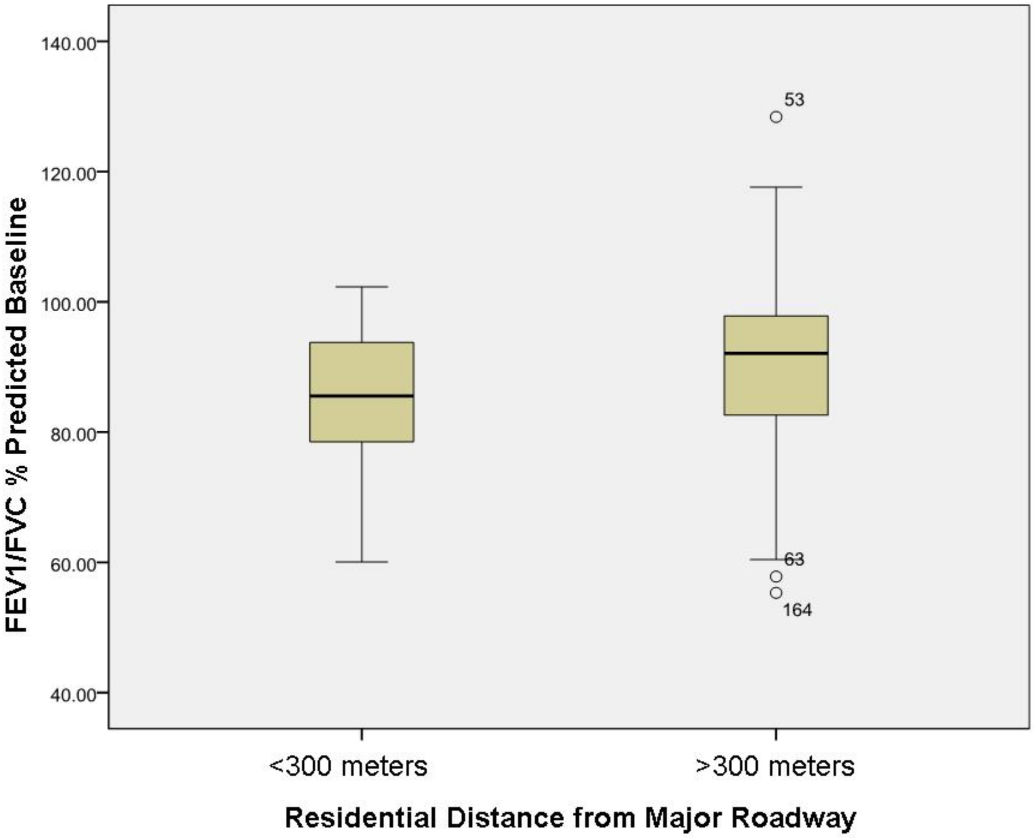


Figure 6.

FEV$_1$/FVC % Predicted Maximum

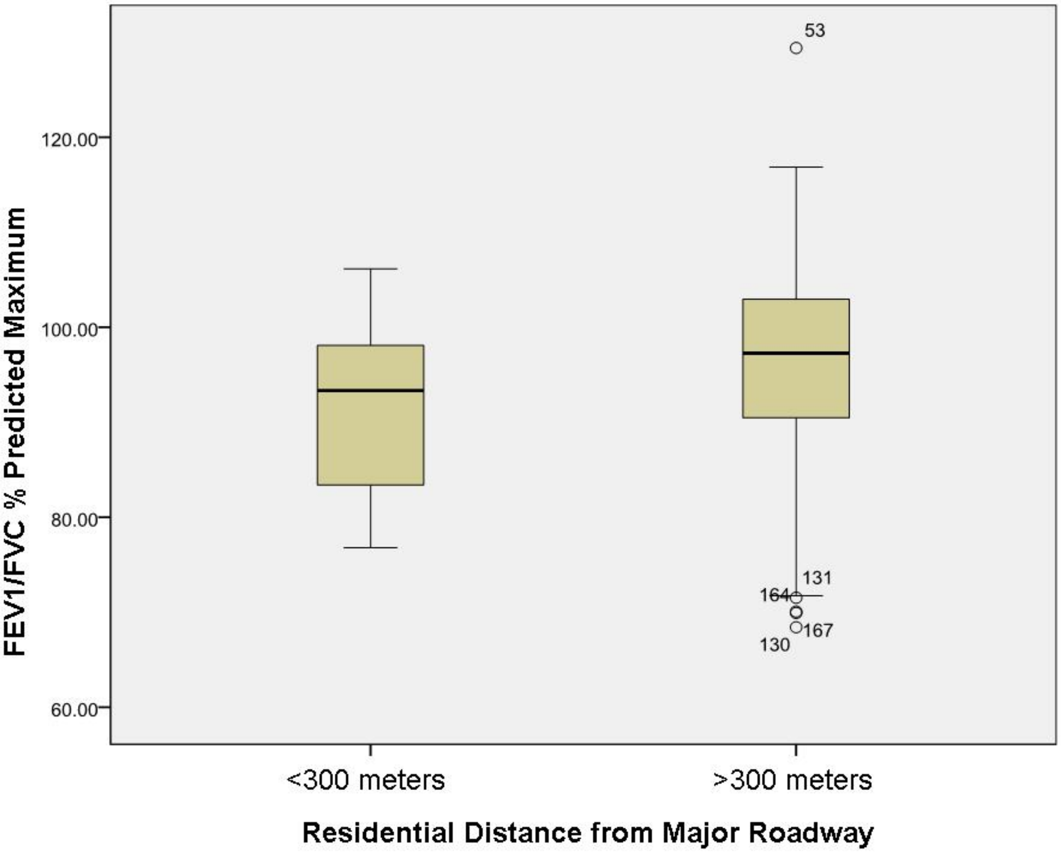
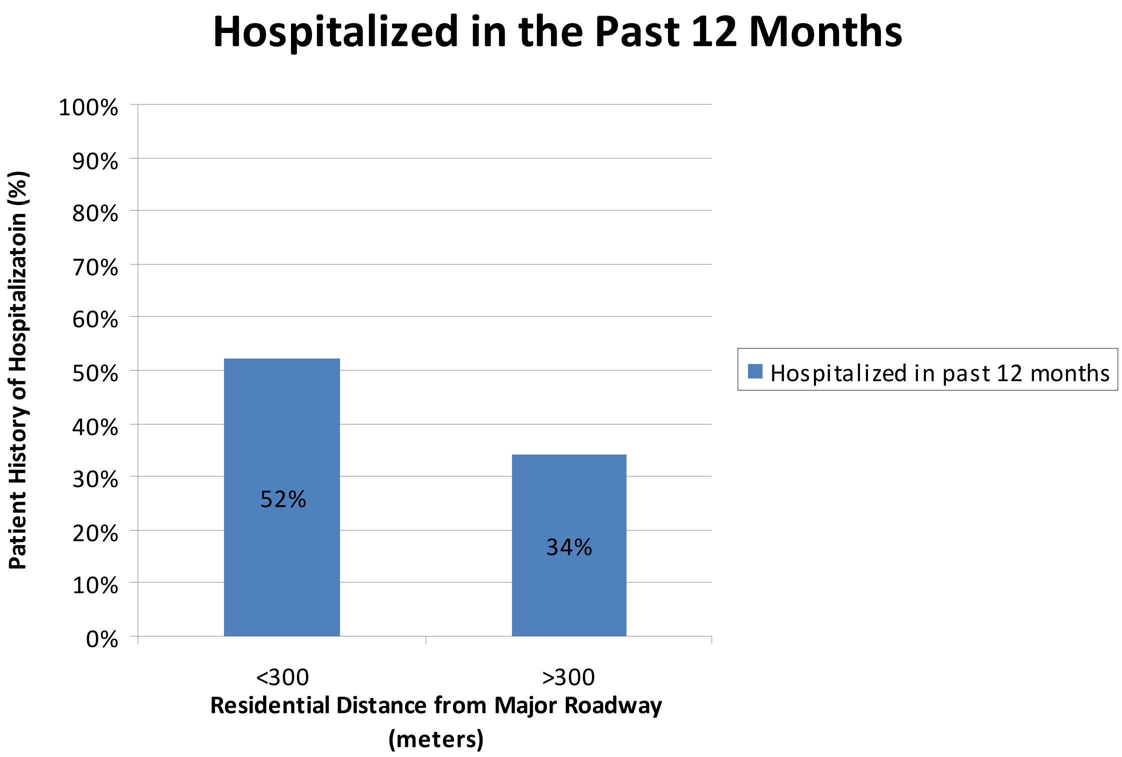


Figure 7.


Hospitalized in the Past 12 Months
Patient History of Hospitalizatoin (%)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
52%
34%
<300
>300
Residential Distance from Major Roadway (meters)
Hospitalized in past 12 months

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