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Pediatric Severe Asthma: Role of Environmental Tobacco Smoke?

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Abstract

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Asthma is the most common chronic lung disease of childhood. While several routes of exposure have been implicated in inducing and complicating asthma, exposure to tobacco smoke has remained at the forefront. My thesis seeks to: 1) determine the prevalence of self-reported environmental tobacco smoke (ETS) exposure in asthmatic children, 2) determine whether plasma cotinine concentrations are higher in asthmatic children with self-reported ETS exposure, and 3) to determine whether plasma cotinine concentrations are associated with clinical features of asthma severity in children. These questions were addressed through database analysis and by testing blood samples for cotinine, which is a biomarker of nicotine. Overall, 20% of all children with asthma were exposed to ETS. Exposure, as identified by cotinine levels, matched self-reported ETS exposure. Cotinine levels did not correlate with asthma severity. Therefore, although ETS exposure is highly prevalent in asthmatic children, these data suggest that ETS exposure alone does not contribute to asthma severity.

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Table of Contents

INTRODUCTION

Severe asthma in children is a complicated disease associated with chronic airway inflammation and ongoing symptoms. 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 manor: "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."[24] FEV_1 is a pulmonary function test that measures the forced expiratory volume in one second that a patient can exhale.[25] 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.[26] FEV1 and other pulmonary function test results are used to diagnose a variety lung diseases including asthma.

In the airways of asthmatic patients, there is increased size and proliferation of the upper airway cells as well as bronchial obstruction and bronchoconstriction.[2] 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.[27] In pediatric severe asthma, these features persist despite treatment with high doses of inhaled corticosteroids.[11] Although the prevalence of severe asthma in the general population is low, roughly 10% of the affected children[3] have severe asthma as well as extreme morbidity which leads to disproportionate health care utilization and a significant economic burden.[4] Of the $19.7 billion spent each year on asthma-related

care, severe asthma accounts for up to 50% of all asthma costs.[3] Severe asthma in children is therefore an important public health problem that warrants further study.

Atlanta, Georgia, was recently named one of the "top 10 asthma capitals" of the United States[5] due to the high prevalence[6] and increased severity[7] of asthma in this area. Indeed, there are a large number of children with severe asthma in Atlanta who are as young as 6 years of age[8]. Inhaled corticosteroids (ICS) are the cornerstone of asthma treatment,[9] but these children with severe asthma have frequent exacerbations despite aggressive treatment with high doses of ICS.[10] To date, the biological and environmental factors associated with severe asthma in children are poorly understood.

Despite asthma's causality not being well known, 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 (described previously) and radiological tests such as chest radiographs or a computed tomography scan.[28] 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.[28] Radiological tests can also reveal lung tissue abnormalities that may be hindering air intake capacity.[28] 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.[28]

Although smoking and chronic environmental tobacco smoke (ETS) exposures are associated with increased mortality rates, ETS exposure of children is still prevalent

and is a major risk factor for poor lung health.[24,13] According to the Third National Health and Nutrition Examination Survey, roughly 38% of all children in the United States are exposed to ETS at home.[2] This exposure prevalence is highest in children of low socioeconomic status, where parents often have low education levels.[26]

There is a clear association between ETS exposure, both prenatal and postnatal, and the development of asthma in children. Babies born to mothers who continued smoking during their pregnancy have an 83-246% increased risk of asthma.[13] Exposure to ETS after birth also increases the risk of a child developing asthma[14] and is particularly damaging for younger children two months to five years of age.[2] In one study, 60% of all asthmatic children were exposed to ETS in their first years of life.[15]

Although the pathophysiology of severe asthma is not well understood, ETS exposure may predispose children to more severe forms of asthma that are very difficult to treat. In comparison to children with low ETS exposure, children with high ETS exposure were more likely to develop moderate or severe asthma with an odds ratio of 2.7.[16] There is also a correlation to dose exposure as the incidence of asthma-like symptoms increases with the number of smokers living in a home.[2] This may be related to the particle size of ETS, which is much smaller than main stream smoke particles, and therefore allows for greater penetrability into the airways.[13] This increased penetrability may ultimately induce asthma symptoms by augmenting the expression and secretion of IL-13, which is an important cytokine of allergic inflammation.[17]

The relationship between ETS exposure and asthma is not limited to the development of the disease. Children with pre-existing asthma who are exposed to ETS

develop further complications including decreased lung function, increased asthma exacerbations, heightened sensitivity to allergens and increased use of emergency devices.[2,17] Even in healthy children, ETS exposure negatively impacts lung function and is associated with a 2.5% reduction in the maximal expiratory flow and a 0.4% reduction in the FEV_1.[15] These effects are likely greater in children with asthma and may account for the severity of the disease. More studies are needed to understand how ETS exposure influences asthma severity in children.

The purpose of this study was to determine whether exposure to ETS is a risk factor for severe versus mild-to-moderate asthma in children. Analysis was performed on samples collected from children previously enrolled and characterized in the National Heart, Lung and Blood Institute's Severe Asthma Research Program (SARP). The following specific aims were tested:

1. Determine the prevalence of parental self-reported ETS exposure of asthmatic children.
2. Determine whether plasma cotinine concentrations were higher in asthmatic children with parental self-reported ETS exposure.
3. Determine whether plasma cotinine concentrations were associated with clinical features of asthma severity in children.

METHODS

This study was conducted using previously collected plasma samples from children with mild-to-moderate and severe asthma enrolled in the Severe Asthma Research Program **(SARP)**. SARP is an ongoing multicenter study that began in 2001. The purpose of SARP is to investigate the clinical and biological attributes of severe versus mild-to-moderate asthma. Through SARP, a definition of severe asthma was proposed along with a Manual of Procedures, which included 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, 75 children with mild-to-moderate asthma and 72 children with severe asthma have been recruited at Emory University. After informed consent, samples of plasma, exhaled breath condensate (EBC) and bronchoalveolar lavage (BAL) were collected for research purposes. These samples are housed in the Emory University Department of Pediatrics and are under the discretion of Dr. Anne Fitzpatrick (Emory SARP PI).

Subject characterization. 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.[11] Spirometry was briefly performed at baseline and 15 minutes after receiving two inhalations of albuterol sulfate (90 μg/inhalation) for the purpose of relieving bronchial spasms. The results fulfilled criteria for reproducibility and were interpreted according to reference standards.[19] Lung volumes were measured with a whole body plethysmograph and

expressed according to reference standards.[20] Broncho-provocation testing was limited to children with a baseline FEV_1 of at least 70% predicted and was performed using 10 concentrations of methacholine from 0 to 25 mg/mL delivered by a Rosenthal dosimeter.[21] Allergy skin prick testing was performed with a standard kit (Multi-Test II) containing extracts of tree pollen, grass pollen, ragweed pollen, weed pollen, dog hair, cat epithelium, alternaria, cladosporidium, aspergillus, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, and cockroaches. For assessment of airway inflammation, exhaled nitric oxide was collected with a reservoir bag at a fixed exhaled flow rate of 0.35 L/s.[22]

Analysis of samples. Cotinine is a metabolite of nicotine and a sensitive marker of ETS exposure. Cotinine was measured in the plasma of asthmatic children previously enrolled and characterized in SARP. Cotinine was measured using a commercially-available competitive, enzyme-linked immunosorbent assay (ELISA) (Calbiotec).[23] A competitive ELISA is "an analytic procedure in which an unlabeled and a labeled analyte competes for sites to bind to a specific protein" where the higher the original antigen concentration is, the lower the measured output signal will be **(Figure 1)**.[37] A competitive ELISA is advantageous when measuring plasma cotinine levels because it allows you to analyze unpurified samples with many other background antigens and still target the antigen of interest. A Calbiotech Cotinine ELISA kit was used to analyze the Cotinine levels in SARP serum samples. The kit was a solid phase competitive ELISA and the wells of the 96-well plate were coated with an antibody derived against cotinine. As per the kit instructions, 10 µl of each concentration of pre-made cotinine standard (0.0, 5.0, 10.00, 25.00, 50.00, 100.00 ng/m) was pipetted in duplicate into a 96-well plate. 10µl of each

plasma sample was pipetted, in singlet, into the 96-well plate. Next, 100 µL of a second cotinine antibody conjugated to horseradish peroxidase enzyme was added to each well. The plate was agitated for 30 seconds to ensure proper mixing. The plate was incubated for one hour at room temperature in the dark. All wells were then washed six times with 300 µL of distilled water using a multi-channel pipette and an augmented plate washer. Following an inversion onto absorbent paper, 100 µL of the chromagenic substrate for the horseradish peroxidase 3,3',5,5'-tetramethylbenzidene reagent was added to each well. The plate was then incubated for 30 minutes in the dark at room temperature for color development. After the 30 minute time period, 100 µL of stop solution was added to each well and then the plate was shaken to ensure complete mixing. The absorbance was then read on a Teacan plate reader at a wavelength of 450 nm. The cotinine values of the samples were interpolated from the standard curve.

Data analysis. Data analysis was performed with SigmaStat software using two-tailed tests of significance and an alpha level of 0.05. Data were graphically inspected and logarithmically transformed when departures from normality were identified. Associations between continuous variables (cotinine and biomarkers of asthma severity) were determined with Pearson bivariate correlations. The overall prevalence of ETS exposure in mild-to-moderate asthmatics versus severe asthmatics was determined using a chi-squared test. A t-test was conducted to compare the difference in cotinine levels among those with ETS exposure and those without. Prior to statistical analysis the data were log-transformed to account for the skew of the results. To determine whether ETS exposure was associated with clinical features of asthma severity, binary logistic

regression was performed using asthma severity (severe vs. mild-to-moderate) as the dependent variable and cotinine as a predictor. Other T-tests were performed using lung function, symptom and allergy data as dependent variables and cotinine as a predictor.

RESULTS

Study Population Demographics

There was no statistical difference in age between mild-to-moderate and severe asthmatics in this study population **(Table 1)**. Nor was there any statistical difference between the number of males with either mild-to-moderate or severe asthma. The severe asthmatics were predominantly African American and were statistically different from the percentage of African Americans in the mild-to-moderate category ($p < 0.05$). Severe asthmatics were statistically significantly younger at the age of diagnosis than the age at diagnosis for the mild-to-moderate asthmatics ($p < 0.05$). Surprisingly, the severe asthmatics did not have more ER visits in the past twelve months when compared to mild-to-moderate asthmatics. However, severe asthmatics were three times more likely to have made a visit to the ER in their lifetime when compared to mild-to-moderate asthmatics ($p < 0.05$). Severe asthmatics were almost six times more likely to have been hospitalized when compared to mild-to-moderate asthmatics ($p < 0.05$). Per the SARP definition, the severe asthmatics baseline percent FEV_1 was significantly lower than the baseline percent FEV_1 of mild-to-moderate asthmatics.

Prevalence of ETS Exposure

The prevalence of ETS exposure among pediatric asthmatics was measured by proxy of plasma cotinine concentrations from blood samples. Based on this analysis, the overall prevalence of ETS exposure among children with pediatric asthma, both mild-to-moderate and severe, in the SARP sample cohort was nearly 20%. In addition, when controlling for the confounders of sex, age, African American ethnicity, age of diagnosis,

number of emergency room visits in the last year, lifetime hospitalizations, and base-line FEV1, the exposure to ETS did not differ between children with mild-to-moderate and severe asthma.

Association between plasma cotinine and self-reported ETS exposure

Assuming a normal cotinine level of zero, fifty-five patients, or roughly 82% of patients had a negative cotinine test and twelve patients, or roughly 18% of patients, had a positive cotinine test. However, some cases had discordant questionnaire answers from the laboratory results regarding ETS exposure. Roughly 12% of all of the asthmatics in this study who reported ETS exposure actually tested cotinine negative. In contrast, 30% of the asthmatics listed no home ETS exposure but tested cotinine positive. The children whose parents self-reported ETS exposure had a mean cotinine concentration of 3.12 μg/mL. Children of parents who self-reported no ETS exposure had a mean cotinine concentration of 0.094 μg/mL which was three folds lower than asthmatics whose parents self-reported ETS exposure **(Figure 2)**.

Association between plasma cotinine and clinical features of asthma severity

In this sample set, cotinine did not correlate with markers of asthma severity in all enrolled children or when asthmatics were separated into groups with mild-to-moderate and severe asthma. When FEV_1 was used as the indicator of lung function, there was no statistical difference between mild-to-moderate asthmatics with ETS exposure and those without ETS exposure **(Figure 3A).** Similarly, there was no difference in FEV_1 in severe asthmatics with or without ETS exposure **(Figure 3B)**. There was no statistical

difference of allergic sensitization as measured by IgE and ETS exposure between mild-to-moderate asthmatics **(Figure 4A)** and severe asthmatics (**Figure 4B)**. When the percentage of eosinophils was used as a measure of allergic sensitization, there was no statistical difference between mild-to-moderate asthmatics with or without ETS exposure **(Figure 5A)** or severe asthmatics with or without exposure (**Figure 5B)**. Likewise, ETS exposure did not significantly alter the total number of hospital visits among mild-to-moderate asthmatics **(Figure 6A)** or severe asthmatics (**Figure 6B)**. For exhaled nitric oxide, a marker of lung inflammation, there was no statistical difference between the exhaled parts per billion (ppb) of nitric oxide in mild-to-moderate asthmatics **(Figure 7A)** or severe asthmatics (**Figure 7B)** with or without ETS exposure. Furthermore, there was no correlation between lifetime hospital visits and ETS exposure among severe asthmatics **(Figure 8A)**. Similarly, there was no correlation between lifetime hospital visits and ETS exposure when children with severe and mild-to-moderate asthmatics were combined.

DISCUSSION

This study found that nearly 20% of all children with asthma in this sample were exposed to ETS as measured by serum cotinine concentrations. There was an association between plasma cotinine concentrations and self reported ETS exposure which demonstrates that cotinine is a sensitive indicator of ETS exposure and was significantly elevated in children exposed to ETS at home. However, exposure to ETS did not differ between children with mild-to-moderate asthma and those with severe asthma. This was supported by no statistical difference in plasma cotinine levels between the two groups. In addition, there was no correlation between plasma cotinine level and several common measurable indicators of asthma severity. Specifically, there was no correlation between cotinine and FEV1, cotinine and lifetime ER visits, cotinine and exhaled nitric oxide, cotinine and eosinophils and no correlation between cotinine and measured IgE.

The finding of this study that roughly 20% of children with asthma in Atlanta were exposed to ETS was consistent with other reports.[36] However, no associations between asthma severity and ETS exposure were observed in this study. Regarding the correlation between asthma severity and cotinine exposure, a prior study by Mannino, Homa and Redd, found that children with greater ETS exposure had a higher odds ratio of developing moderate or severe asthma. However, that study did not mention a further stratification with an increased odds of developing severe asthma over mild-to-moderate asthma which is supported by the findings of this paper.[16] The finding that plasma cotinine concentrations are a sensitive indicator of ETS exposure and correlate with children who are exposed to ETS in the home supports the findings of many other studies

that reported that cotinine measures correlated with ETS exposure evaluations on questionnaires.[16, 30, 31]

This study was limited by the number of samples available for cotinine testing. Therefore, some of the statistical tests were under powered and may have resulted in an underestimation of the association between plasma cotinine concentrations and asthma severity. Another limitation of this study is that the definition of asthma is not defined by concrete variables but rather a diagnosis is made based on a number of variable attributes which makes that outcome hard to quantify. This study might also have been limited by the sensitivity of the cotinine assay or the short half life of this nicotine metabolite, which is roughly 24 hours in vivo, or by self-reporting of the patients. Cotinine analysis is only indicative of ETS exposure in the past 24 hours and does not reveal information about past exposures. Therefore, a negative cotinine test might not be an accurate measure of long-term ETS exposure but rather acute exposure. For example, roughly 12% of all of the asthmatics in this study reported ETS exposure but tested cotinine negative while 30% of the asthmatics listed no home ETS exposure but tested cotinine positive. This discordant response could indicate either untruthful questionnaires responses or that there are significant ETS exposures that the parent is unaware of. However, this exposure misclassification was nondifferential between mild-to-moderate and severe asthmatics. As discussed above, prior studies suggested that the development of asthma was associated with ETS exposure in the first years of life.[13,14, 2,15] In the current study, we only examined the effects of current ETS exposure and cannot rule out the possibility that asthma severity may be related to the ETS exposure within the first years of life rather than current exposure.

In summary we have shown that roughly 20% of asthmatics in this cohort were exposed to ETS but that increasing amounts of ETS exposure do not correlate with increased severity of asthma and that cotinine did not correlate with markers of asthma severity. These data suggest that severe asthma in children is more complex and is not solely due to ETS exposure in the home. Thus, more research is needed to understand how the severity outcome of asthma is determined. One possible explanation for this negative finding is that while ETS might not have an acute impact on asthma severity, it could be an underlying long-term systemic problem that impacts asthma severity by way of DNA degradation. In particular, future studies should focus on the aspect DNA degradation and oxidative stress and should include analysis of markers of DNA degradation and oxidative stress such as histone deacetylase, 8-hydroxy-2'-deoxyguanosine (8-OhdG), thiocyanates, protein carbonyls and malondialdehyde.

Despite increased knowledge regarding the health effects of ETS exposure, nearly one out of every five children in the Atlanta metropolitan area are exposed to ETS at home. Additional studies are needed to understand how ETS exposure affects the development and severity of asthma in children. Other markers of asthma severity might be needed to better classify asthma patients. Additional public health interventions to decrease smoking rates are warranted, particularly in parents of school-age children with asthma.

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APPENDIX

Table 1.

	Mild-to-Moderate Asthmatics (n = 35)	**Severe Asthmatics (n = 33)**
Age (in years)	10.40 ± 3.02	10.64 ± 2.94
Males	22 (63%)	16 (48%)
African American	20 (57%)	28 (85%)**
Age when diagnosed	3.88 ± 3.70	1.42 ± 1.73**
ER visits (past year)	0.44 ± 0.50	0.70 ± 0.47
ER visits (ever)	1.53 ± 2.36	3.42 ± 3.79**
Hospitalizations (ever)	0.94 ± 1.89	5.58 ± 5.27**
Baseline FEV_1 (%)	98.21 ± 15.82	80.2 ± 17.32**
Exhaled Nitric Oxide (ppb)	22.83 ± 18.54	32.09 ± 34.35
Eosinophils (%)	5.02 ± 3.52	7.53 ± 6.42
IgE (kU/L)	396.71 ± 625.26	633.71 ± 735.08

Data are shown as the mean ± standard deviation or the frequency percentage. **Groups are significantly different at the $p > 0.05$ level.

Figure Legends

Figure 1.

Competitive solid phase ELISA assay. The diagram depicts the mechanism involved in a direct competitive solid phase ELISA that measures cotinine concentrations in plasma. Each well is coated with an anti-cotinine antibody and contains a sample and a cotinine enzyme conjugate, in this case horseradish peroxidase (HRP). Within the well, the cotinine in the sample competes with the HRP for cotinine binding sites. When the substrate is added, the residual cotinine binds the substrate and changes color where the intensity of the color inversely related to concentration. This can be read on a plate reader and determines the concentration of cotinine present.

Figure 2.

Smoke exposure correlates with plasma cotinine levels in this sample. Plasma cotinine levels were higher in asthmatic children with parental self-reported ETS exposure. The average plasma cotinine concentration in those who had self-reported ETS exposure was 3.12 ng/mL with a standard error of 6.20 while those who self-reported no ETS exposure had an average plasma cotinine concentration of 0.09 ng/mL with a standard error of 0.26. The bar represents the mean plasma cotinine concentration. Standard error is represented by the black line extending from the top of each vertical bar. There was good agreement between self-reported ETS exposure and ETS exposure verified by cotinine analysis. This indicates that plasma cotinine levels are a good marker of ETS exposure in children with asthma.

Figure 3A.

Relationship between percent FEV_1 and ETS exposure in children with mild-to-moderate asthma. 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.[33] Among children with mild-to-moderate asthma, those with no ETS exposure had an average FEV_1 of 94.95 with a standard error of 2.98 and those with ETS exposure had an average FEV_1 of 106.75 with a standard error of 6.62. The bar represents the mean but the standard error is represented by the black line extending from the top of each vertical bar. The average FEV_1 for children with mild-to-moderate asthma not exposure to ETS is not statistically different than the average FEV_1 for children exposed to ETS.

Figure 3B.

Relationship between percent FEV_1 and ETS exposure in children with severe asthma. Among children with severe asthma, those with no ETS exposure had an average FEV_1 of 82.45 with a standard error of 4.10 and those with ETS exposure had an average FEV_1 of 75.60 with a standard error of 4.70. The bar represents the mean and the standard error is represented by the black line extending from the top of each vertical bar. The average FEV_1 for children with mild-to-moderate asthma not exposure to ETS is not statistically different than the average FEV_1 for children exposed to ETS.

Figure 4A.

Relationship between log-transformed IgE and ETS exposure in children with mild-to-moderate asthma. IgE is used as an indicator of asthma severity because it gauges allergic sensitization which can be the cause of an asthma attack and the more severe the IgE reaction test the more severe that asthma diagnosis.[34] Among children with mild-to-moderate asthma, those with no ETS exposure had an average IgE of 5.04 with a standard error 0.31 and those with ETS exposure had an average IgE of 4.92 with a standard error of 0.60. The bars respresent the mean and the standard error is represented by the black line extending from the top of each vertical bar. The average IgE for children with mild-to-moderate asthma not exposure to ETS is not statistically different than the average IgE for children exposed to ETS.

Figure 4B.

Relationship between log-transformed IgE and ETS exposure in children with mild-to-moderate asthma. Among children with mild-to-moderate asthma, those with no ETS exposure had an average IgE of 5.04 with a standard error of 0.31 and those with ETS exposure had an average IgE of 4.92 with a standard error of 0.45. The bar represents the mean and the standard error is represented by the black line extending from the top of each vertical bar. The average IgE for children with mild-to-moderate asthma not exposure to ETS is not statistically different than the average IgE for children exposed to ETS.

Figure 5A.

Relationship between log-transformed percent eosinophils and ETS exposure in children with mild-to-moderate asthma. Percent eosinophil count is used as an indicator of asthma severity because it indicates allergic sensitization measured by a white blood cell count.[35] The greater the percentage of eosinophils the more severe the allergic sensitization and the more severe asthma diagnosis will be. Among children with mild-to-moderate asthma, those with no ETS exposure had an average percent eosinophil count of 1.65 with a standard error of 0.18 and those with ETS exposure had an average percent eosinophil count of 1.57 with a standard error of 0.18. The bar represents the mean and the standard error is represented by the black line extending from the top of each vertical bar. The average percent of eosinophils for children with mild-to-moderate asthma not exposure to ETS is not statistically different than the average eosinophil count for children exposed to ETS.

Figure 5B.

Relationship between log-transformed percent eosinophils and ETS exposure in children with mild-to-moderate asthma. Among children with severe asthma, those with no ETS exposure had an average percent eosinophil count of 1.85 with a standard error of 0.21 and those with ETS exposure had an average percent eosinophil count of 1.69 with a standard error of 0.26. The bar represents the mean and the standard error is represented by the black line extending from the top of each vertical bar. The average percent of eosinophils for children with severe asthma not exposure to ETS is not statistically different than the average eosinophil count for children exposed to ETS.

Figure 6A.

Relationship between lifetime ER visits and ETS exposure in children with mild-to-moderate asthma. Among children with mild-to-moderate asthma, those with no ETS exposure had an average of 1.46 ER visits in their lifetime with a standard error of 0.50 and those with ETS exposure had an average of 1.80 ER visits in their lifetime with a standard error of 0.79. The bar represents the mean and the standard error is represented by the black line extending from the top of each vertical bar. The average number of lifetime ER visits for children with mild-to-moderate asthma not exposure to ETS is not statistically different than the average number of lifetime ER visits for children exposed to ETS.

Figure 6B.

Relationship between lifetime ER visits and ETS exposure in children with severe asthma. Among children with severe asthma, those with no ETS exposure had an average of 5.44 ER visits in their lifetime with a standard error of 0.99 and those with ETS exposure had an average of 5.90 ER visits in their lifetime with a standard error of 2.08. The bar represents the mean and the standard error is represented by the black line extending from the top of each vertical bar. The average number of lifetime ER visits for children with severe asthma not exposure to ETS is not statistically different than the average number of lifetime ER visits for children exposed to ETS.

Figure 7A.

Relationship between log-transformed exhaled nitric oxide and ETS exposure in children with mild-to-moderate asthma. Exhaled nitric oxide is used to diagnosis asthma severity as an indicator of airway inflammation and the higher the ppb the more severe the asthma diagnosis.[36,11] Among children with mild-to-moderate asthma, those with no ETS exposure had an average exhaled nitric oxide level of 2.79 ppb with a standard deviation of 0.90 and those with ETS exposure had an average exhaled nitric oxide level of 2.77 with a standard deviation of 0.85. The bar represents the mean and the standard error is represented by the black line extending from the top of each vertical bar. The average exhaled nitric oxide level for children with mild-to-moderate asthma not exposed to ETS is not statistically different than the average exhaled nitric oxide level for children exposed to ETS.

Figure 7B.

Relationship between log-transformed exhaled nitric oxide and ETS exposure in children with severe asthma. Among children with severe asthma, those with no ETS exposure had an average exhaled nitric oxide level of 2.79 ppb with a standard error of 0.23 and those with ETS exposure had an average exhaled nitric oxide level of 2.77 with a standard error of 0.03. The bar represents the mean and the standard error is represented by the black line extending from the top of each vertical bar. The average exhaled nitric oxide level for children with severe asthma not exposed to ETS is not statistically different than the average exhaled nitric oxide level for children exposed to ETS.

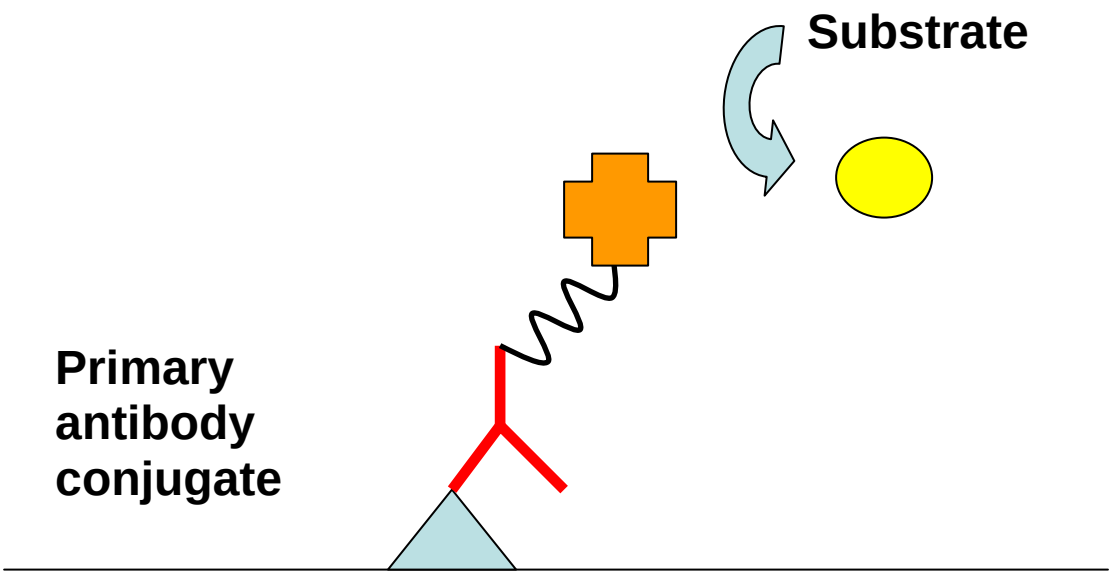
Figure 8A.

Relationship between log-transformed plasma cotinine concentrations and lifetime ER visits among all asthmatics with mild-to-moderate and severe asthmatics combined. Each dot represents either a mild-to-moderate or severe asthmatic. The line descending through the data represents a linear regression and shows that there is no correlation between cotinine and lifetime ER visits when all asthmatics are combined.

Figure 8B.

Relationship between log-transformed plasma cotinine concentrations and lifetime ER visits only among children with severe asthma. Each dot represents a severe asthmatic. The line descending through the data represents a linear regression and shows that there is no correlation between cotinine and lifetime ER visits among severe asthmatics which corresponds with the lack of trend between cotinine and lifetime ER visits among all asthmatics combined.

Figure 1.



Direct Competition Assay

Figure 2.

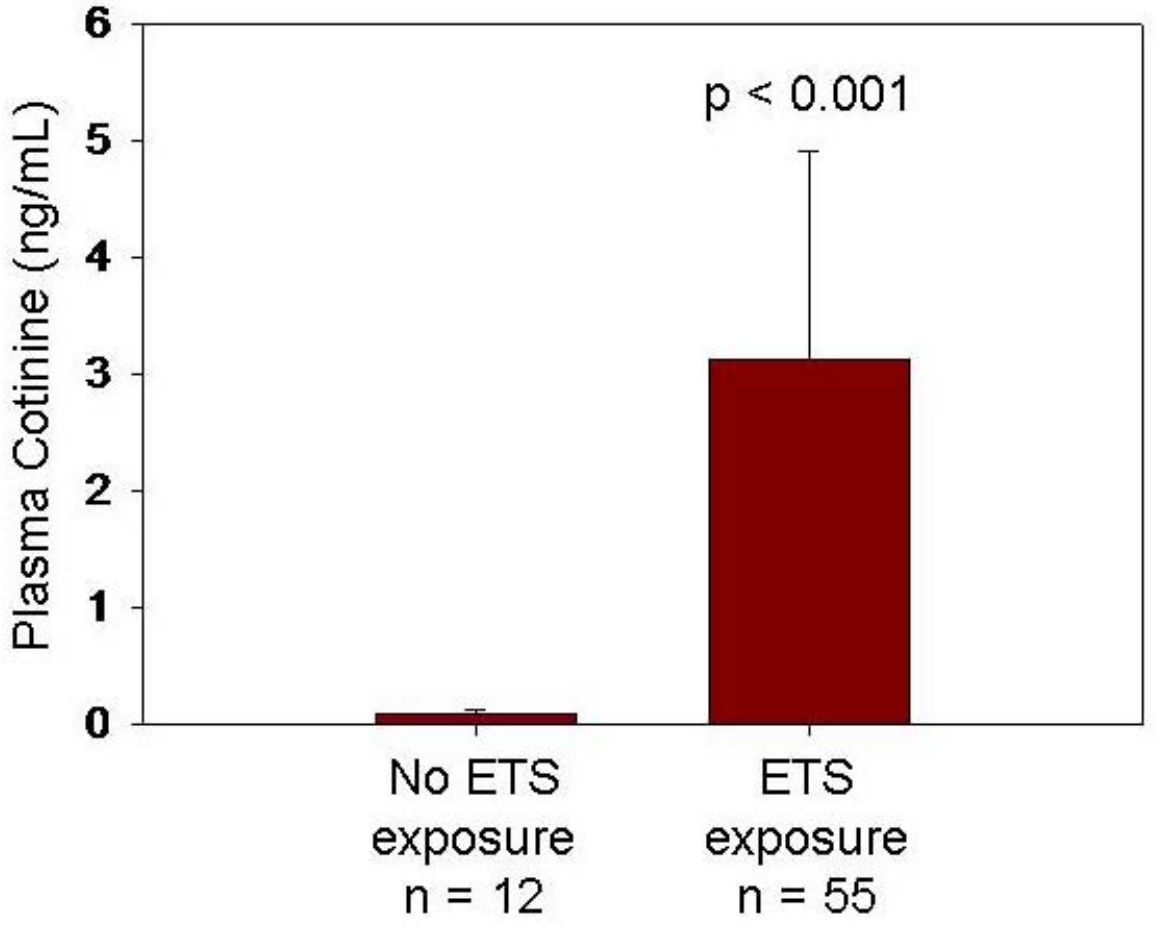

Plasma Cotinine (ng/mL)
6
5
4
3
2
1
0
p < 0.001
No ETS exposure n = 12
ETS exposure n = 55

Figure 3.

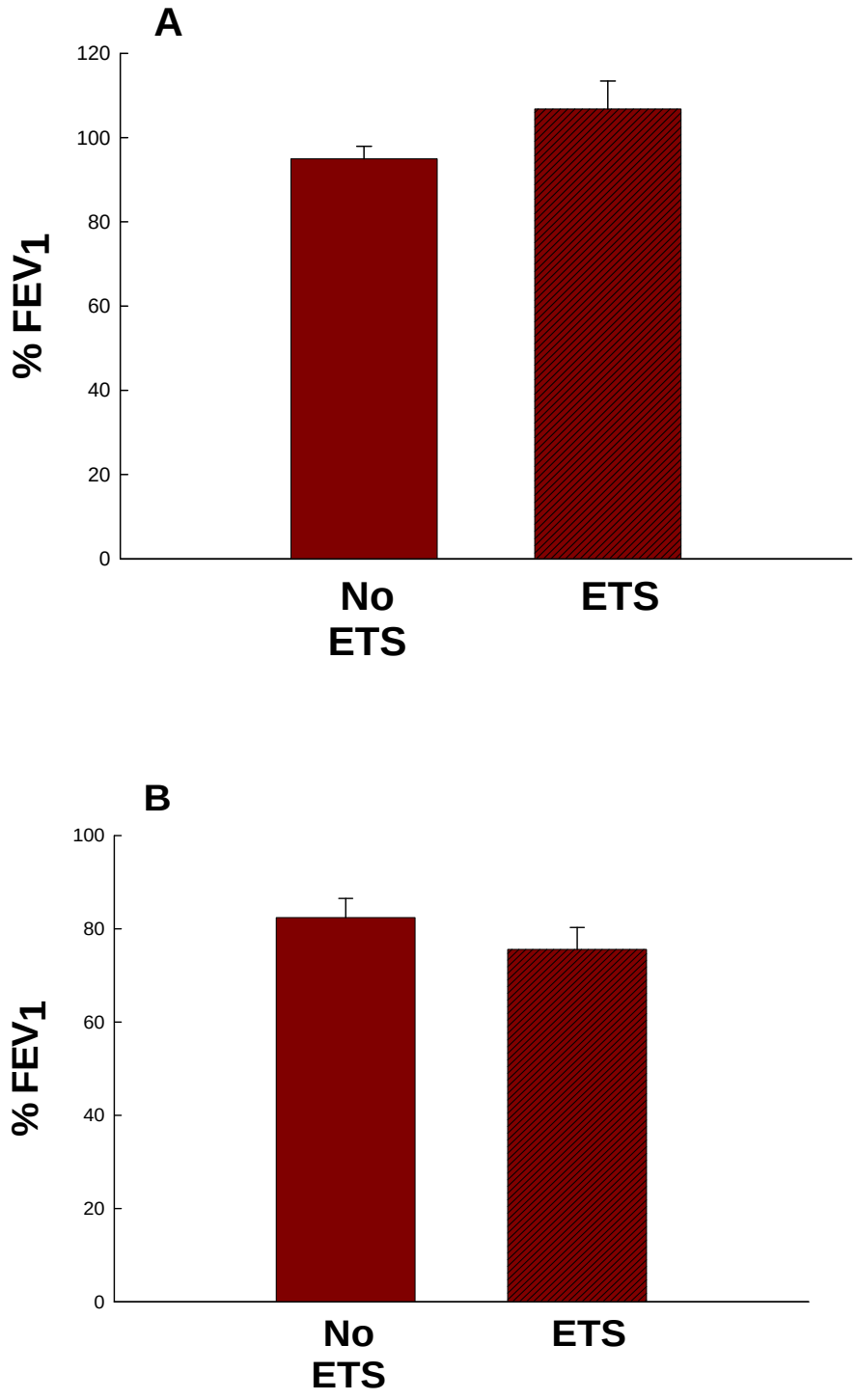

A
120
100
80
60
40
20
0
% FEV1
No ETS
ETS
B
100
80
60
40
20
0
% FEV1
No ETS
ETS

Figure 4.

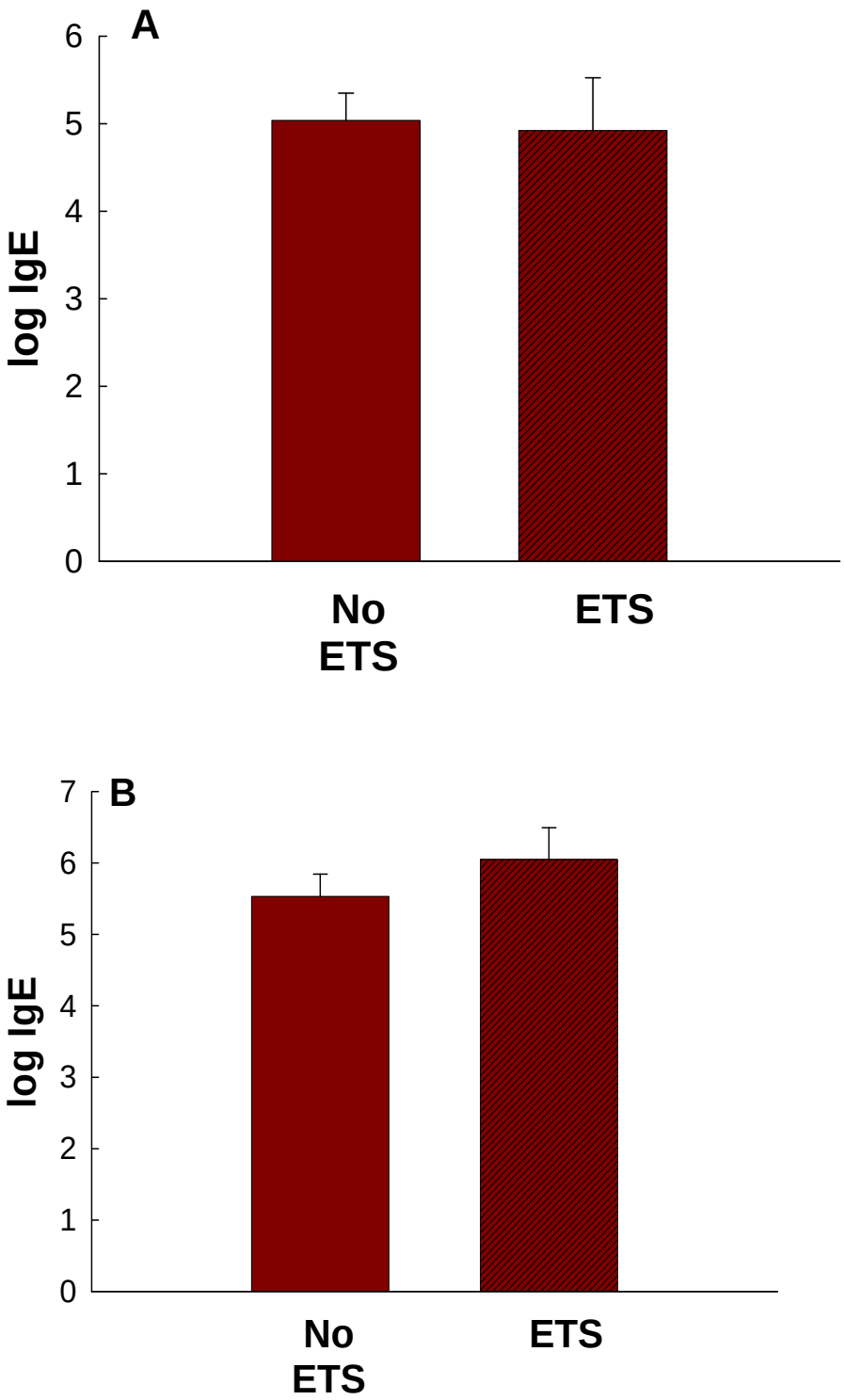
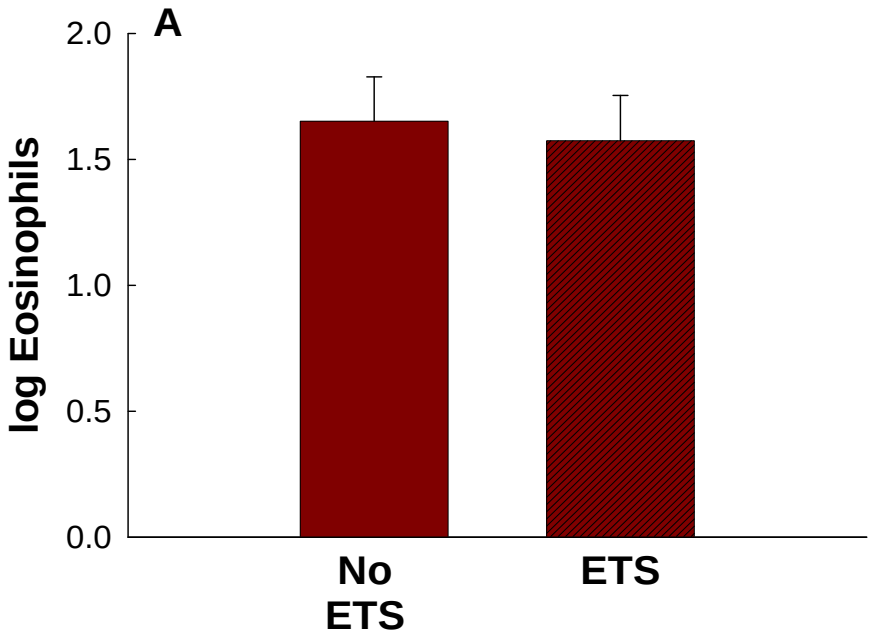


Figure 5.


A
2.0
1.5
1.0
0.5
0.0
log Eosinophils
No ETS
ETS

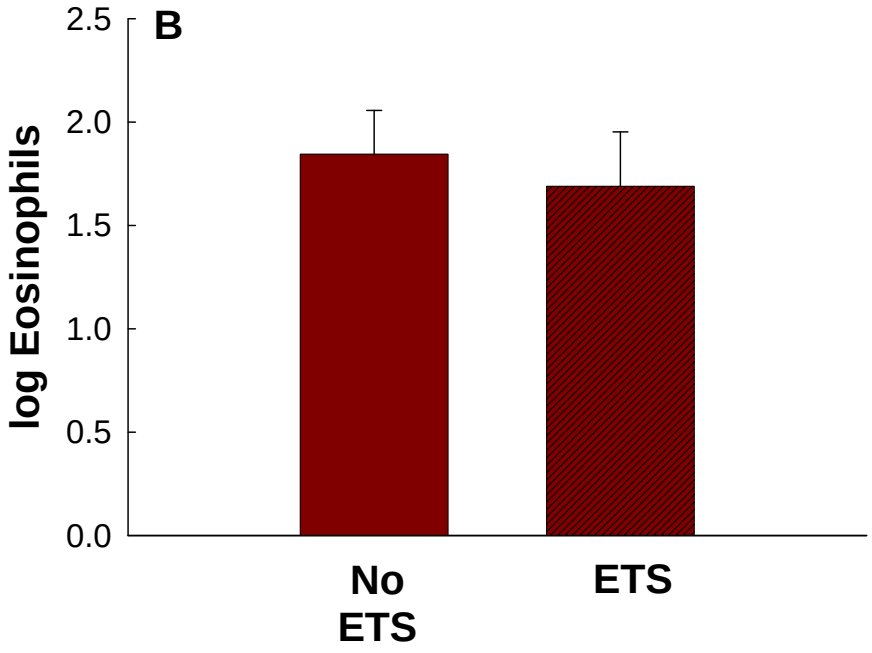

B
2.5
2.0
1.5
1.0
0.5
0.0
log Eosinophils
No ETS
ETS

Figure 6.

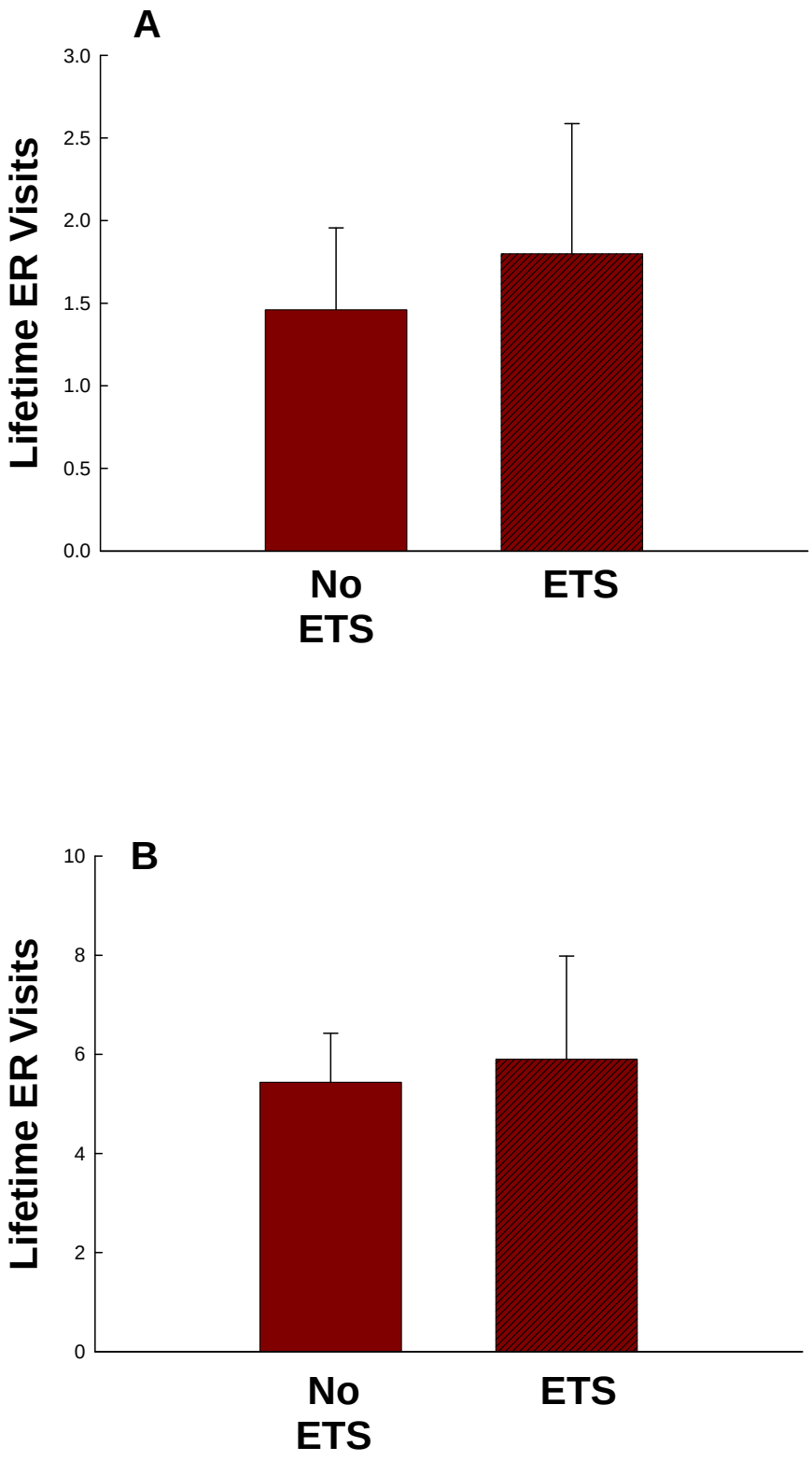

A
Lifetime ER Visits
3.0
2.5
2.0
1.5
1.0
0.5
0.0
No ETS
ETS
B
Lifetime ER Visits
10
8
6
4
2
0
No ETS
ETS

Figure 7.

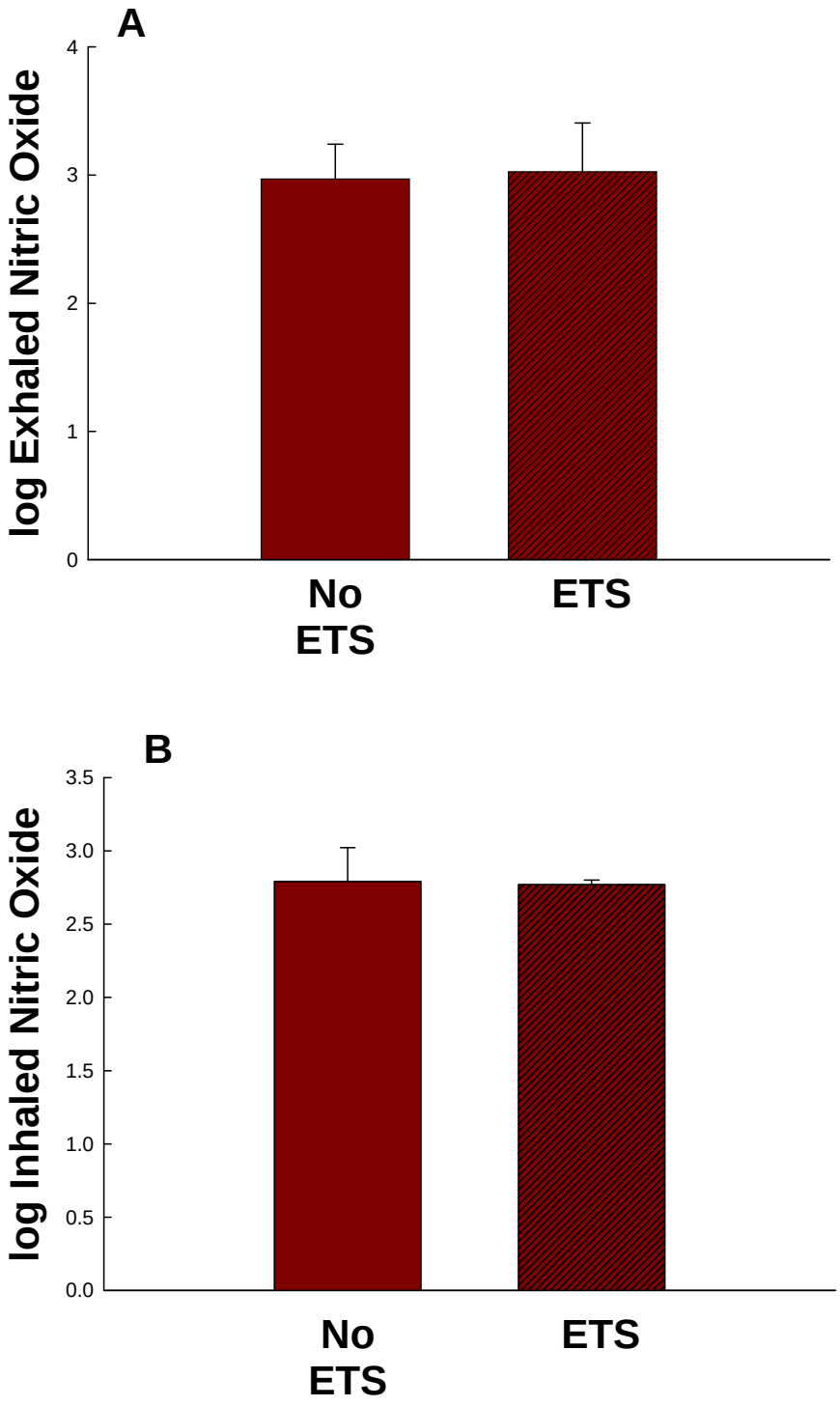
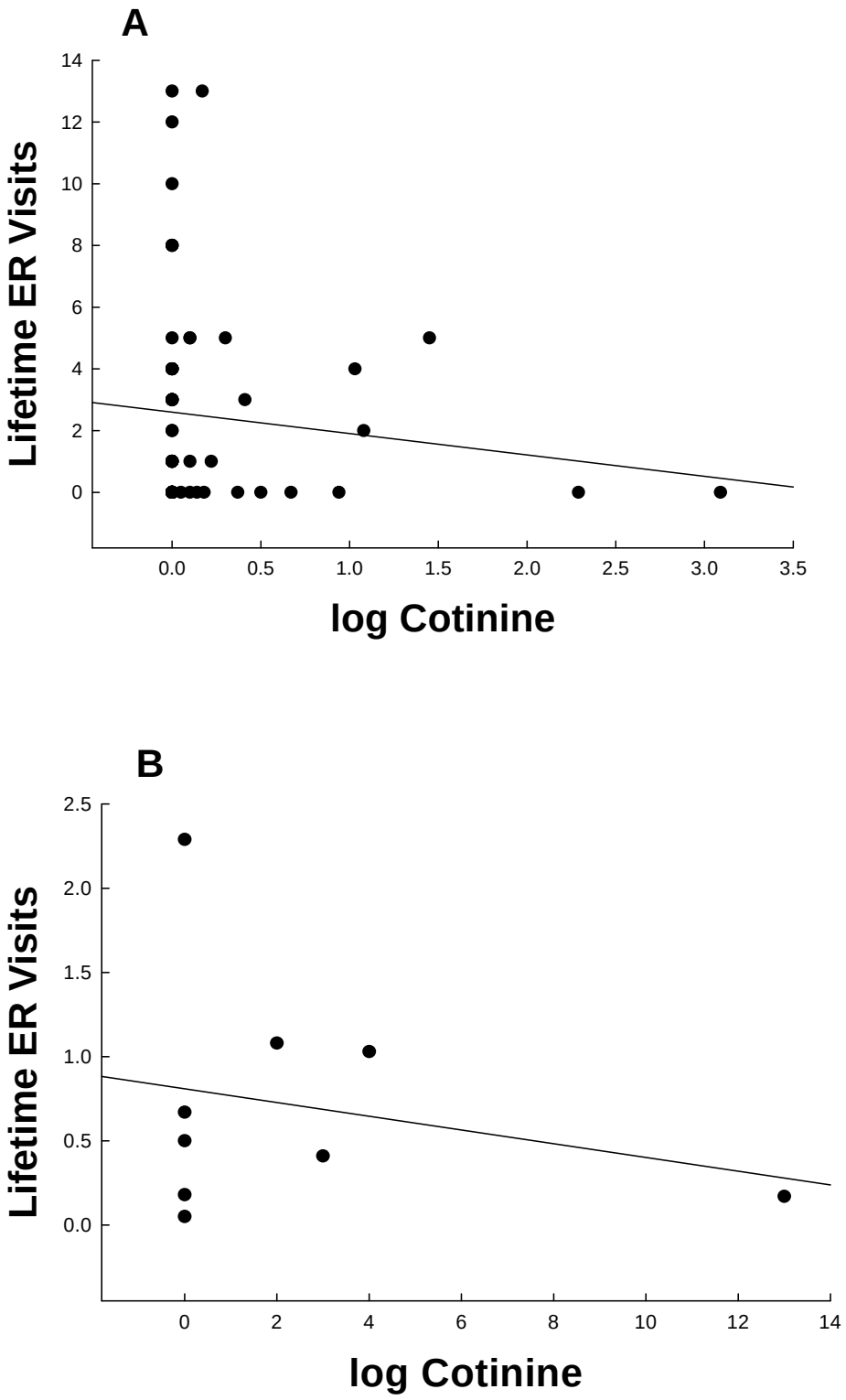

A
log Exhaled Nitric Oxide
4
3
2
1
0
No ETS
ETS
B
log Inhaled Nitric Oxide
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
No ETS
ETS

Figure 8.


A
Lifetime ER Visits
14
12
10
8
6
4
2
0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
log Cotinine
B
Lifetime ER Visits
2.5
2.0
1.5
1.0
0.5
0.0
0
2
4
6
8
10
12
14
log Cotinine