# Exposure to Traffic-related Particles and Endotoxin during Infancy Is Associated with Wheezing at Age 3 Years

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*Rationale*: **Murine models demonstrate a synergistic production of reactive oxygen species on coexposure to diesel exhaust particles and endotoxin.**

*Objectives*: **It was hypothesized that coexposure to traffic-related particles and endotoxin would have an additive effect on persistent wheezing during early childhood.**

*Methods*: **Persistent wheezing at age 36 months was assessed in the Cincinnati Childhood Allergy and Air Pollution Study, a high-risk birth cohort. A time-weighted average exposure to traffic-related particles was determined by applying a land-use regression model to the homes, day cares, and other locations where children spent time from birth through age 36 months. Indoor levels of endotoxin were measured from dust samples collected before age 12 months. The relationship between dichotomized (</≥75th percentile) traffic-related particle and endotoxin exposure and persistent wheezing, controlling for potential covariates, was examined.**

*Measurements and Main Results*: **Persistent wheezing at age 36 months was significantly associated with exposure to increased levels of traffic-related particles before age 12 months (OR = 1.75; 95% confidence interval, 1.07–2.87). Coexposure to endotoxin had a synergistic effect with traffic exposure on persistent wheeze (OR = 5.85; 95% confidence interval, 1.89–18.13) after adjustment for significant covariates.**

*Conclusions*: **The association between traffic-related particle exposure and persistent wheezing at age 36 months is modified by exposure to endotoxin. This finding supports prior toxicological studies demonstrating a synergistic production of reactive oxygen species after coexposure to diesel exhaust particles and endotoxin. The effect of early versus later exposure to traffic-related particles, however, remains to be studied because of the high correlation between exposure throughout the first 3 years of life.**

**Keywords:** particles; diesel; land-use regression; wheeze; endotoxin

### AT A GLANCE COMMENTARY

#### Scientific Knowledge on the Subject

Prior studies suggest that exposure to diesel exhaust particles (DEP) results in the production of reactive oxygen species (ROS). Previous studies of traffic-related pollution and wheezing during childhood have not examined exposure during early infancy to traffic-related particles in combination with indoor endotoxin, which may lead to increased persistent wheezing in at-risk children.

#### What This Study Adds to the Field

This study provides evidence that exposure to traffic-related particles is associated with childhood wheeze and that a synergistic relationship exists between coexposure to traffic-related particles and endotoxin during infancy and persistent wheezing at age 3 years.

Toxicological and epidemiologic studies have demonstrated a consistent association between exposure to air pollution and exacerbation of existing asthma (1, 2). Proximity to major roads, a surrogate of traffic exposure, has been associated with decreased lung growth (3), increased asthma symptoms (4), increased airway inflammatory markers including exhaled nitric oxide (5), and increased oxidative stress markers (6).The association between exposure to air pollution and development of asthma, however, is less clear, although recent research has found incident asthma to be associated with exposure to traffic-related air pollution (7). Air pollution in urban areas is a complex mixture of particles and gas-phase pollutants arising from a myriad of sources. The association between traffic-related air pollution and respiratory health effects in children is of interest due to the toxicological effects of the air pollution mixture arising from mobile sources (i.e., gasoline and diesel combustion engines) (8). In particular, fine and ultrafine particulate matter (PM2.5 and PM0.1, respectively), is derived primarily from vehicular exhaust and, in contrast to PM10, has a larger fraction of elemental and organic carbon (9). Diesel exhaust particles (DEP), a model particulate air pollutant, are a major component of PM2.5, particularly in urban areas where diesel exhaust is the largest single source of airborne PM from vehicles (10, 11). As such, DEP have been widely studied with respect to adverse respiratory health effects (10); it has been demonstrated that they are associated with increased inflammatory cells, increased cytokine levels, decreased macrophage function, and increased airway resistance (10). Although the mechanisms by which DEP exert their toxicological effects remain unknown, the heterogeneous mixture of diesel exhaust is likely associated with the generation of reactive oxygen species (ROS) and inflammation. Laboratory studies have shown DEP to also have immune adjuvant properties, enhancing production of allergen-specific IgE (12–16) and production of Th2 cytokines, including IL-4, IL-5, IL-10, and IL-13 (12, 17).

Animal and human studies have shown that inhalation of endotoxin induces airway inflammation (18, 19), and the proinflammatory effect of endotoxin is enhanced by concomitant

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exposure to DEP (20). High exposure to endotoxin has been associated with wheezing in children (21). A murine experimental model has shown that coexposure to DEP and endotoxin are additive in forming oxygen free radicals in lungs (18), resulting in enhanced neutrophilic lung inflammation and proinflammatory cytokines (22). Paradoxically, endotoxin may also suppress cytokine production after stimulation by DEP (23).

Exposure to DEP and endotoxin either alone or in combination may modify immune responses early in life and may be important in the subsequent development of allergic respiratory disorders in childhood. At birth, the infant immune system is biased toward Th2 immune responses that can initially be manifested in the first 2 years of life by atopic dermatitis and food allergies (24, 25). It has been hypothesized that failure of the immune system to modify or suppress Th2-biased cellular cytokine responses can lead to development of atopic clinical phenotypes, including allergic rhinitis and asthma. Thus, there may be periods of life, particularly in the first year, when air pollutants, indoor endotoxin, pets, and aeroallergen exposures may either modify or enhance development of allergic disorders in childhood (26).

The Cincinnati Childhood Allergy and Air Pollution Study (CCAAPS) is a prospective birth cohort study of children born to atopic parents in the greater Cincinnati metropolitan area (27). A previous report found that infants exposed to high levels of traffic-related particles were significantly more likely to have parental reported wheezing without a cold before age 1 (28). This follow-up study examines estimated levels of exposure to traffic-related particles and coexposure to indoor endotoxin. The central hypothesis of this study was that traffic-related particle exposure during early childhood increases the risk of persistent wheezing at age 3 years, and this effect is modified by exposure to indoor endotoxin. Some of these studies have previously been reported in the form of an abstract (29).

## METHODS

### Study Population

Detailed information regarding the study's objectives, recruitment methods, air monitoring, and protocols is available (27, 30, 31). Children enrolled in the study were identified from birth records. Infants were eligible for study recruitment if their residence at time of birth was either less than 400 m from a major road (defined as $\geq$ 1,000 trucks/d) or more than 1,500 m from a major road. Additionally, all enrolled infants had at least one atopic parent confirmed by symptoms and skin prick test (SPT) to 15 aeroallergens (27).

Children were clinically evaluated annually at ages 1, 2, and 3 years, receiving an SPT and physical examination. Parents were administered a questionnaire gathering information on parental and child health in the previous year and environmental exposures, including environmental tobacco smoke (ETS) and pets. History of locations where the child spent 8 or more hours per week (e.g., home, day care, relative's home) from birth through age 3 years were collected. Children who had completed the clinical examination at age 36 months were included in this report.

A home visit was conducted before the child's first birthday (average age of 8 mo) and included a detailed environmental assessment and house dust sample collection. House dust samples were collected from the floor of the child's primary activity room identified by the parents as the room where the child spent most of his/her daytime. Participants were requested not to clean the floor for at least 1 day before dust sampling. The majority of infants spent their daytime in either the living room (56%) or the family room (36%). Dust samples were collected using a vacuum cleaner (Filter Queen Majestic; HMI Industries, Inc, Seven Hills, OH) at a flow rate of 800 L/min. A custom-made cone-shaped high-efficiency particulate air filter trap (Midwest Filtration, Cincinnati, OH) was attached to the nozzle of the vacuum cleaner. All home visits and dust samples were conducted by trained teams (32). After collection, dust samples were stored desiccated at $-20^{\circ}$C until further analysis.

Endotoxin concentrations were determined by the limulus amebocyte lysate test (Associates of Cape Cod Inc, Falmouth, MA) in all samples according to methods described by Milton and colleagues (33). All glassware and materials used were endotoxin- and pyrogen-free. Intra-assay mean coefficient of variation (CV) ranged from 2.1 to 4.6% (SD, 1.5–4.2), and the interassay mean CV was 16.1% (SD, 9) (34). A total of 37 samples were below the lower limit of detection (6 endotoxin units/mg of dust). Endotoxin levels below the LOD were analyzed as LOD/2 (34, 35).

### Health Outcomes

*Recurrent wheezing* at age 36 months was defined as parental report of two or more wheezing episodes in the previous 12 months at the 36-months clinic visit. *Persistent wheezing* at age 36 months was designated if the child was reported by his/her parent to have had two or more wheezing episodes in the previous 12 months at both their 36- and 24-months clinic visit. A child was also considered to have persistent wheezing at age 36 months if the parent reported at the 36-months visit that his/her child had been diagnosed with asthma by their private physician. *Persistent allergic wheezing* required a positive SPT to at least one aeroallergen at age 36 months, whereas *persistent nonallergic wheeze* was defined as a negative SPT to all aeroallergens at age 36 months.

Children were also classified as having increased risk of future asthma based on an Asthma Predictive Index (API) proposed by Castro-Rodriguez and colleagues (36) and modified by Guilbert and colleagues (37). Children were considered to have a positive API if they were reported to have recurrent wheezing at age 36 months and met at least one of three major criteria (parental asthma history, allergic sensitization to one or more aeroallergen, and eczema) or two of three minor criteria (wheezing without a cold, physician-diagnosed allergic rhinitis, and allergic sensitization to milk or egg). Eczema was defined as either physician-diagnosed eczema on physical examination or parental report of frequent skin scratching for more than 6 months accompanied by red spots, raised bumps, or rough, dry, scaly skin.

### Traffic-related Particle Exposure

Each participating child's average daily exposure to traffic-related particles was calculated using a land-use regression model as described in Ryan and colleagues (38). Ambient air sampling was conducted at a total of 27 sampling sites in the greater Cincinnati area from December 2001 through December 2006. The average daily level of sampled elemental carbon attributable to traffic (ECAT), a marker of traffic-related particles, was determined for each sampling site as described in the online supplement. Regression models were developed to relate this marker of traffic-related particles measured at the 27 sampling sites with land-use and traffic variables. The final land-use regression model had an $R^2$ equal to 0.73 and contained independent variables related to elevation, truck intensity within 300 m, length of bus routes within 300 m, and wind direction (38).

Individual traffic exposure was calculated as a child's time-weighted average daily exposure during the following periods: birth to 6 months, 7 to 12 months, 13 to 24 months, and 25 to 36 months. This exposure metric was determined by first geocoding all addresses where the child was reported to have spent more than 8 hours per week within each time period and deriving a time-weighted microenvironmental exposure estimate (38). All geocoding and geographic information systems were conducted using EZLocate (TeleAtlas, Lebanon, NH) and ArcGIS 9.0 (Environmental Systems Research Institute, Redlands, CA).

### Statistical Analysis

The correlation and distribution of average daily exposure to ECAT was examined for each time period (0–6, 7–12, 13–24, 25–36 mo). Exposure to ECAT and endotoxin were highly skewed (Figure 1) and subsequently dichotomized using the 75th percentile (average daily exposure to ECAT $\geq$/$<$0.41 $\mu g/m^3$) to define high/low exposure. Univariate analyses were conducted to assess the association between environmental exposures (ECAT, ETS, endotoxin, visible mold in the home) and potential covariates (race, household income, sex, parental history of asthma, day care attendance, report of an upper respiratory condition in the previous 12 months, report of a lower respiratory condition in the

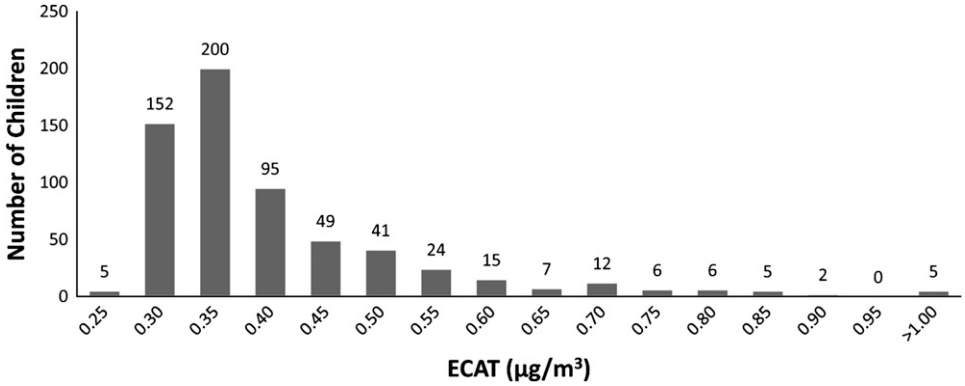


*Figure 1.* Distribution of estimated traffic-related particle exposure. ECAT = elemental carbon attributable to traffic.

previous 12 months, breast-feeding) with recurrent wheeze, persistent wheeze, and asthma predictive index at age 36 months. ETS exposure was categorically defined as parental report of at least one current smoker residing in the household at age 36 months. Parental history of asthma (yes/no) was determined by parental report of either the biological mother or biological father ever having been diagnosed by a physician with asthma. Upper respiratory conditions (yes/no) were defined as parental report of at least one of the following: ear infection, sinus infection, strep throat, tonsillitis, colored drainage in the previous 12 months reported at the 36-months visit. Lower respiratory condition (yes/no) was defined as parental report of at least one of the following: whooping cough, croup, viral infections, bronchitis/bronchiolitis, respiratory flu, or pneumonia in the previous 12 months reported at the 36-months visit. Visible mold was categorically defined as present/absent based on the home visit before age 12 months if any sign of visible mold was observed in the home. Breastfeeding was dichotomized (yes/no) by parental report of breast-feeding for 4 weeks or more after birth. First-order interaction products between environmental exposures were also examined. Environmental exposures, covariates, and first-order interactions significant at the 10% level ($P < 0.1$) were initially included in multivariate logistic regression models for each outcome. As race and income were significantly correlated ($P < 0.01$), race was selected for consideration in the multivariate models. Multivariate models included, *a priori*, exposure to ECAT and endotoxin. Additional significant environmental exposures, covariates, and first-order interactions remaining in each final multivariate model were chosen using backward elimination with variables remaining in the model having a $P$ value less than 0.1.

## RESULTS

The CCAAPS cohort enrolled 762 children at age 1 year. Of these, 82% (n = 624) completed the age 3 years clinical examination, questionnaire, and SPT and were included in this analysis. The average age of the child at the time of the age 3 study visit was 36.6 (SD, 2.3) months. The prevalence of persistent wheezing, recurrent wheezing, and positive asthma predictive index was 13% (n = 82), 17% (n = 103), and 16% (n = 97), respectively. Of those children defined as having persistent wheeze at age 36 months (n = 82), persistent wheezing was defined by parental report of wheezing episodes for 83% (n = 68) and personal physician diagnosis for 17% (n = 14). Of those children with a positive asthma predictive index, 87% (n = 71) were reported to have persistent wheezing at age 3 years. Of those with persistent wheezing, 42 had a concurrent positive SPT (persistent allergic wheeze), whereas the rest (n = 40) had nonallergic persistent wheezing. Children who completed the age 3 years clinic visit were similar to those who did not with respect to sex, race, and parental history of asthma but were less likely to have ETS exposure and an annual household income less than $20,000 ($P < 0.05$).

Indoor endotoxin values were available for 77% (n = 483) of the 624 children completing the age 3 years examination. Of these 483, the prevalence of persistent wheezing was 14% (n = 66) (16 subjects reporting persistent wheeze were removed from analyses examining endotoxin due to lack of indoor endotoxin data). Children having indoor endotoxin measurements were significantly more likely to be white, have a household income greater than or equal to $20,000 per year, and be breast-fed, and less likely to be exposed to ETS ($P < 0.05$). There were no significant differences in this subset with respect to sex, parental history of asthma, day care attendance, upper respiratory conditions, lower respiratory conditions, and prevalence of persistent wheezing.

The mean average daily exposure to ECAT at ages 6, 12, 24, and 36 months was 0.39 (SD, 0.14), 0.39 (SD, 0.14), 0.38 (SD, 0.14), and 0.38 (SD, 0.12) $\mu g/m^3$, respectively, and was significantly correlated throughout each time period (Table 1). Therefore, further analyses were conducted using the average daily ECAT exposure during the first 12 months of life as this likely represents a critical time period of development and corresponds to the time of endotoxin exposure assessment.

The prevalence of persistent wheeze, recurrent wheeze, and positive asthma predictive index was examined by quartile of exposure (Table 2). The univariate association between persistent wheeze, recurrent wheeze, and asthma predictive index was significantly elevated only among children exposed to the highest quartile of average daily exposure to ECAT ($\geq$/$<$0.41 $\mu g/m^3$) (Table 2). Subsequent analyses used this dichotomization to define high/low exposure (i.e., $\geq$/$<$75th percentile of average daily ECAT exposure).

Subject characteristics and the results of univariate analyses are presented in Table 3. Significant univariate associations were observed between all wheezing outcomes and ECAT exposure (Table 3). Exposure to ETS, parental history of asthma, sex, race, and respiratory infections (upper and lower) were associated with all outcomes and considered for inclusion in the multivariate model (Table 3). Significant first-order interactions were observed for ECAT and endotoxin exposure with persistent wheezing and a positive asthma predictive index (Table 3). The effect modification of ECAT exposure on persistent wheeze, recurrent wheeze, and asthma predictive index by high levels of endotoxin in the home is illustrated in Figure 2. Among children exposed to low levels of endotoxin in the home, the

**TABLE 1. CORRELATION OF AVERAGE DAILY ELEMENTAL CARBON ATTRIBUTABLE TO TRAFFIC EXPOSURE BY CHILD AGE**

| | 0–6 mo | 7–12 mo | 13–24 mo | 25–36 mo |
|---|---|---|---|---|
| 0–6 mo | 1 | | | |
| 7–12 mo | 0.93 | 1 | | |
| 13–24 mo | 0.86 | 0.94 | 1 | |
| 25–36 mo | 0.70 | 0.72 | 0.81 | 1 |

**TABLE 2. PREVALENCE OF PERSISTENT WHEEZE, RECURRENT WHEEZE, AND POSITIVE ASTHMA PREDICTIVE INDEX AT AGE 36 MONTHS BY QUARTILE OF ECAT EXPOSURE**

| Exposure | | Persistent Wheeze | | Recurrent Wheeze | | Asthma Predictive Index* | |
|---|---|---|---|---|---|---|---|
| Quartile ECAT | Estimated ECAT ($\mu g/m^3$) | % Wheeze | OR† (95% CI) | % Wheeze | OR† (95% CI) | % Positive | OR† (95% CI) |
| <25th percentile | ⩽ 0.30 | 9.0 | 1 | 13.5 | 1 | 12.2 | 1 |
| ⩾25th percentile to 50th percentile | 0.31–0.34 | 12.2 | 1.4 (0.7–2.9) | 14.1 | 1.1 (0.6–2.0) | 12.8 | 1.1 (0.5–2.1) |
| ⩾50th percentile to 75th percentile | 0.35–0.40 | 12.8 | 1.5 (0.7–3.1) | 16.7 | 1.3 (0.7–2.4) | 14.1 | 1.2 (0.6–2.3) |
| ⩾75th percentile | ⩾0.41 | 18.6 | 2.3 (1.2–4.6) | 21.8 | 1.8 (1.0–3.3) | 23.1 | 2.2 (1.2–4.0) |

*Definition of abbreviations*: CI = confidence interval; ECAT = elemental carbon attributable to traffic; OR = odds ratio.

* Asthma Predictive Index: Defined as positive if the child was reported to have recurrent wheezing at age 36 months and met at least one of three major criteria (parental asthma history, allergic sensitization to more than one aeroallergen, and eczema) or two of three minor criteria (wheezing without a cold, physician-diagnosed allergic rhinitis, and allergic sensitization to milk or egg).

† Unadjusted odds ratios.

prevalence of persistent wheeze was 11% in those exposed to low levels of ECAT and 18% among children exposed to high levels of ECAT. In the presence of high endotoxin in the home, the prevalence of persistent wheeze remained the same when exposed to low levels of ECAT (11%) but significantly increased to 36% among children coexposed to high levels of endotoxin and ECAT (Figure 2).

The results of the final multivariate logistic regression models are presented in Table 4. After backward elimination, exposure to ETS, parental history of asthma, sex, and having had a lower respiratory condition in the previous 12 months remained significant in the final model (Table 4) for persistent wheeze. The interaction between ECAT and endotoxin exposure also remained significant after adjustment. The association between persistent wheeze and exposure to ECAT was significantly increased in the presence of high endotoxin (OR = 5.85; 95% CI, 1.89–18.13) when compared with those with low ECAT and endotoxin (Table 4). To examine the sensitivity of the dichotomized ECAT exposure, the continuous estimates of ECAT exposure and endotoxin (log-transformed) were entered into the model in lieu of the categorized variables and the interaction between endotoxin and ECAT remained significant ($\beta = 0.94$; $P = 0.06$). Significant associations were also observed between recurrent wheeze at age 36 months and ETS, parental history of asthma, sex, and lower respiratory conditions, although ECAT, endotoxin, and the interaction between ECAT and endotoxin did not remain significant in the final model. Exposure to ECAT, ETS, visible mold in the home, sex, and lower respiratory conditions were significant in the multivariate model with asthma predictive index, though the interaction between ECAT and endotoxin was not (Table 4).

The associations between persistent allergic and nonallergic wheezing and ECAT were also examined (*see* Table E1 in the online supplement). Persistent allergic wheeze was associated, although not significantly, with exposure to high ECAT (OR = 2.11; 95% CI, 0.97–4.61), in comparison to children without persistent allergic wheeze (i.e., no current wheezing or current wheezing without current allergic sensitization) after adjustment for endotoxin, sex, parental asthma, race, lower respiratory conditions, and breast-feeding. The interaction between ECAT and endotoxin did not remain significant in the final multivariate model. In the multivariate model examining children with persistent nonallergic wheeze in comparison to children without persistent nonallergic wheeze (i.e., no current wheezing or current wheezing and a positive SPT) a significant effect modification was observed between high ECAT and endotoxin (OR = 3.76; 95% CI, 1.01–14.03) after adjustment for sex, parental history of asthma, and lower respiratory conditions.

## DISCUSSION

This study found a significant association between exposure to traffic-related particles in the first year of life and persistent wheeze at age 3 years. Furthermore, to our knowledge, this report is the first to follow up on the animal and human experimental studies (17, 19) relating combined exposure to traffic particles and endotoxin with respiratory effects on a cohort of children. A synergistic interaction between estimated traffic-related particle exposure and endotoxin in the home resulted in increased persistent wheezing, particularly nonallergic, at age 36 months. These results support the hypothesis proposed by Yeatts and colleagues (39) that diverse environmental exposures (e.g, air pollutants, ETS, indoor contaminants, aeroallergens, viral infections) may exert combined effects on the airways occurring at different time points throughout life, ultimately determining clinical outcomes.

Endotoxin is a component of the cell wall of gram-negative bacteria and likely stimulates the maturing immune system to develop Th1-type immune responses. Although some studies have shown that exposure to endotoxin or surrogates of endotoxin is protective against the development of allergic disease in children (40, 41), others have found that endotoxin increases risk of wheezing during early childhood (42–44). Celedon and colleagues (45) recently demonstrated that exposure to endotoxin before age 1 year was inversely associated with the development of atopy. In this same high-risk cohort, however, exposure to high levels of endotoxin during infancy increased the risk for development of asthma and late-onset wheezing at age 7 years (45). Previously in this birth cohort we reported that exposure to both multiple dogs and high endotoxin during infancy was protective for wheeze at age 1 year (34). The effect of either exposure alone, however, was not significant. In the current study, the definition of persistent wheeze at age 3 years applies to older children with recurrent wheezing reported over a time period of at least 2 years. The apparent conflicting results between the aforementioned studies related to effects of endotoxin exposure may be due to how the outcomes are defined, different environmental exposures, the population being studied (high-risk atopic vs. a general population), and gene–environment interactions. In addition, as endotoxin exposure was assessed before age 1 year, it is not known the current level of endotoxin exposure in the home.

Although persistent wheeze was not significantly associated with endotoxin exposure alone, coexposure to endotoxin in the presence of high traffic-related particle exposure resulted in increased risk (Table 4). Interestingly, although persistent allergic and nonallergic wheeze were significantly associated with traffic-related particle exposure, the effect modification of

**TABLE 3. UNADJUSTED ASSOCIATION BETWEEN ENVIRONMENTAL EXPOSURES, ENVIRONMENTAL FIRST-ORDER INTERACTIONS, AND POTENTIAL COVARIATES WITH PERSISTENT WHEEZING, CURRENT WHEEZING, AND ASTHMA PREDICTIVE INDEX AT AGE 36 MONTHS**

| | | OR (95% CI) | | |
|---|---|---|---|---|
| Exposure/Covariate | n (%) | Persistent Wheeze | Recurrent Wheeze | Asthma Predictive Index* |
| ECAT | | | | |
| High† | 158 (25) | 1.75 (1.07–2.87) | 1.58 (1.00–2.49) | 1.96 (1.24–3.10) |
| Low | 466 (75) | 1 | 1 | 1 |
| Endotoxin‡ | | | | |
| High | 121 (25) | 1.25 (0.71–2.22) | 1.26 (0.74–2.14) | 1.52 (0.90–2.57) |
| Low | 362 (75) | 1 | 1 | 1 |
| ETS§ | | | | |
| Present | 118 (19) | 1.83 (1.08–3.12) | 2.02 (1.25–3.28) | 2.10 (1.28–3.43) |
| Absent | 506 (81) | 1 | 1 | 1 |
| Visible mold in the home | | | | |
| Present | 315 (50) | 1.37 (0.86–2.19) | 1.46 (0.95–2.23) | 1.49 (0.96–2.30) |
| Absent | 309 (50) | 1 | 1 | 1 |
| Parental history of asthma | | | | |
| Yes | 213 (34) | 3.42 (2.12–5.52) | 2.17 (1.42–3.34) | 4.41 (2.80–6.96) |
| No | 411 (66) | 1 | 1 | 1 |
| Sex | | | | |
| Male | 337 (54) | 1.88 (1.15–3.07) | 1.49 (0.96–2.30) | 1.62 (1.04–2.54) |
| Female | 287 (46) | 1 | 1 | 1 |
| Race | | | | |
| African-American | 140 (22) | 1.74 (1.05–2.90) | 1.06 (0.64–1.75) | 1.60 (0.99–2.59) |
| White | 484 (78) | 1 | 1 | 1 |
| Income | | | | |
| ⩽ $20,000 | 98 (16) | 1.62 (0.91–2.88) | 1.27 (0.73–2.20) | 1.74 (1.02–2.97) |
| > $20,000 | 526 (84) | 1 | 1 | 1 |
| Day care attendance | | | | |
| Yes | 208 (36) | 0.93 (0.56–1.54) | 0.99 (0.62–1.56) | 0.94 (0.58–1.51) |
| No | 363 (64) | 1 | 1 | 1 |
| Upper respiratory condition‖ | | | | |
| Yes | 305 (49) | 1.57 (0.98–2.51) | 1.82 (1.18–2.80) | 1.38 (0.89–2.13) |
| No | 319 (51) | 1 | 1 | 1 |
| Lower respiratory condition¶ | | | | |
| Yes | 176 (28) | 2.38 (1.48–3.84) | 3.43 (2.22–5.31) | 2.48 (1.59–3.88) |
| No | 448 (72) | 1 | 1 | 1 |
| Breast-feeding** | | | | |
| Yes | 300 (48) | 0.65 (0.40–1.04) | 0.77 (0.50–1.17) | 0.58 (0.37–0.91) |
| No | 324 (52) | 1 | 1 | |
| ECAT/endotoxin | | | | |
| High/high | 24 (5) | 4.43 (1.72–11.37) | 1.82 (0.63–5.21) | 3.38 (1.29–8.87) |
| High/low | 82 (17) | 1.74 (0.89–3.39) | 1.74 (0.93–3.24) | 2.18 (1.17–4.08) |
| Low/high | 97 (20) | 0.97 (0.47–2.00) | 1.37 (0.74–2.53) | 1.61 (0.86–3.00) |
| Low/low | 280 (58) | 1 | 1 | 1 |
| ECAT/ETS | | | | |
| High/present | 44 (7) | 2.12 (0.92–4.91) | 2.12 (0.98–4.56) | 3.05 (1.46–6.38) |
| High/absent | 112 (18) | 2.08 (1.16–3.71) | 1.84 (1.07–3.16) | 2.08 (1.20–3.60) |
| Low/present | 75 (12) | 2.32 (1.20–4.48) | 2.54 (1.40–4.59) | 2.31 (1.24–4.32) |
| Low/absent | 393 (63) | 1 | 1 | 1 |
| Endotoxin/ETS | | | | |
| High/present | 24 (5) | 1.88 (0.60–5.91) | 2.98 (1.15–7.76) | 4.11 (1.61–10.46) |
| High/absent | 97 (20) | 1.51 (0.78–2.93) | 1.23 (0.66–2.31) | 1.49 (0.80–2.79) |
| Low/present | 58 (12) | 2.89 (1.45–5.76) | 2.38 (1.23–4.61) | 2.68 (1.37–5.22) |
| Low/absent | 304 (63) | 1 | 1 | 1 |

*Definition of abbreviations*: CI = confidence interval; ECAT = elemental carbon attributable to traffic; ETS = environmental tobacco smoke; OR = odds ratio.

* Defined as positive if the child was reported to have recurrent wheezing at age 36 months and met at least one of three major criteria (parental asthma history, allergic sensitization to more than one aeroallergen, and eczema) or two of three minor criteria (wheezing without a cold, physician-diagnosed allergic rhinitis, and allergic sensitization to milk or egg).

† High ECAT: ⩾75th percentile (0.41 $\mu g/m^3$); low ECAT: <75th percentile.

‡ Endotoxin values available for 483 homes.

§ ETS present is defined as parental report of at least one current smoker residing in the household.

‖ Upper respiratory conditions defined as ear infection, sinus infection, tonsillitis, strep throat.

¶ Lower respiratory condition defined as whooping cough, croup, viral infection, bronchitis/bronchiolitis, pneumonia, respiratory flu.

** Parental report of breast-feeding ⩾4 wk.

endotoxin was found with respect to persistent nonallergic wheeze. These results are consistent with previous studies showing that inhalation of endotoxin elicits airways inflammation and increases airway hyperresponsiveness to histamine (46, 47). Braun-Fahrlander and colleagues (40) reported that endotoxin exposure in school-aged children was protective for atopic wheeze but increased the risk of nonatopic wheeze.

Endotoxin also induces inflammation in the lung and generates free radicals (18). In murine models the combined exposure to DEP and endotoxin has been shown to enhance neutrophilic

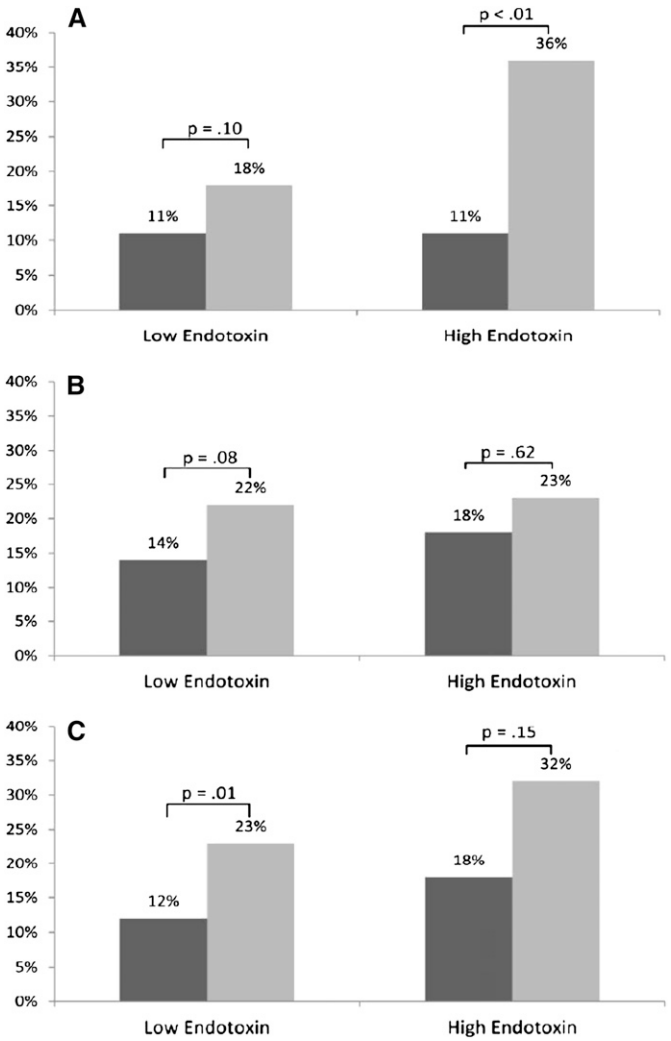


***Figure 2.*** (*A*) Prevalence of persistent wheeze, (*B*) recurrent wheeze, and (*C*) positive asthma predictive index by elemental carbon attributable to traffic (ECAT) and endotoxin exposure. *Solid bars* = low ECAT (<75th percentile); *shaded bars* = high ECAT (⩾75th percentile [0.41 μg/m$^3$]).

**TABLE 4. MULTIVARIATE MODELS FOR PERSISTENT WHEEZE, RECURRENT WHEEZE, AND ASTHMA PREDICTIVE INDEX**

| | OR (95% CI) | | |
|---|---|---|---|
| Exposure/Covariate | Persistent Wheeze | Recurrent Wheeze | Asthma Predictive Index* |
| ECAT[†] | | | |
| High | —[‡] | 1.59 (0.88–2.87) | 2.04 (1.15–3.63) |
| Low | | 1 | 1 |
| Endotoxin | | | |
| High | —[‡] | 1.27 (0.72–2.26) | 1.67 (0.95–2.92) |
| Low | | 1 | 1 |
| ETS[§] | | | |
| Present | 2.14 (1.07–4.27) | 2.47 (1.35–4.52) | 2.31 (1.26–4.21) |
| Absent | 1 | 1 | 1 |
| Visible mold in the home | | | |
| Present | 1.68 (0.92–3.08) | —[‖] | 1.77 (1.04–3.03) |
| Absent | | | 1 |
| Parental history of asthma | | | |
| Yes | 4.03 (2.25–7.23) | 2.12 (1.25–3.57) | —[¶] |
| No | 1 | 1 | |
| Sex | | | |
| Male | 2.78 (1.51–5.11) | 2.05 (1.20–3.50) | 1.83 (1.08–3.10) |
| Female | 1 | | 1 |
| Lower respiratory condition | | | |
| Yes | 3.31 (1.82–5.99) | 3.91 (2.33–6.58) | 2.61 (1.54–4.44) |
| No | 1 | 1 | 1 |
| Breast-feeding | | | |
| Yes | 0.60 (0.33–1.09) | —[‖] | 0.62 (0.36–1.06) |
| No | 1 | | 1 |
| ECAT/endotoxin | | | |
| High/high | 5.85 (1.89–18.13) | —[‖] | —[‖] |
| High/low | 1.43 (0.68–3.02) | | |
| Low/high | 0.88 (0.41–1.91) | | |
| Low/low | 1 | | |

For definition of abbreviations, *see* Table 3.

[†] High ECAT: ⩾75th percentile (0.41 μg/m$^3$); low ECAT: <75th percentile.

* Defined as positive if the child was reported to have recurrent wheezing at age 36 months and met at least one of three major criteria (parental asthma history, allergic sensitization to more than one aeroallergen, and eczema) or two of three minor criteria (wheezing without a cold, physician-diagnosed allergic rhinitis, and allergic sensitization to milk or egg).

[‡] Odds ratios presented for interaction.

[‖] Not included in final multivariate model.

[¶] Not included due to incorporation into outcome definition.

lung inflammation and work synergistically to promote formation of ROS (15, 19). In school-age children with asthma, McConnell and colleagues (48) reported that among children who owned a dog (a potential surrogate of endotoxin exposure) the odds ratio for increased bronchitis symptoms per 4.2 ppb increase in $NO_2$ exposure was 1.49 (95% CI, 1.14–1.95) compared with an odds ratio of 1.16 (95% CI, 0.84–1.60) among children with no dog in the home. In the current study, the prevalence of persistent wheeze among children exposed to high levels of ECAT but low levels of endotoxin was 18%. The prevalence, however, increased to 36% among children exposed to high levels of traffic-related particles with concurrent exposure to high levels of endotoxin (Figure 2).

The findings were similar for both persistent wheezing and a positive API (Table 3). As childhood asthma is difficult to diagnose, the API was used as a marker of risk for future asthma diagnosis. The API was initially developed for children at age 3 years and had a positive predictive value for asthma at ages 6 to 13 of 59%, a negative predictive value of 73%, a specificity of 85%, and a sensitivity of 42% (36). Persistent wheeze and a positive API were associated with ECAT exposure in the multivariate models. Exposure to ECAT, however, was not associated with recurrent wheeze at age 3 years. This finding may be a result of transient wheezing, as opposed to persistent wheezing over the course of at least 2 years, due to factors including viral infections.

A possible limitation of this study is the estimate of elemental carbon attributable to traffic derived from the total sampled elemental carbon in ambient PM2.5. Exposure to ECAT is likely correlated with exposure to other traffic-related air pollution (e.g., $NO_2$). Furthermore, the causative agent in the mixture of air pollution exposure to which children are likely exposed is unknown and may include volatile gases, polycyclic aromatic hydrocarbons (PAHs), and other constituents of air pollution. ECAT is a representative marker of traffic-related particles derived from gasoline and diesel combustion. However, elemental carbon in fine particulate matter is predominately derived from diesel combustion with approximately 75% of diesel PM2.5 comprised of elemental carbon. In contrast, in the eastern United States, approximately 4% of the total fine particulate matter composition is composed of elemental carbon (49). Particulates produced from the combustion of diesel fuel are composed of an elemental carbon core with more than 450 organic compounds attached that are

proposed to be primarily responsible for the proinflammatory and adjuvant effects observed with DEP exposure (10). Future source apportionment research using eight temperature-resolved organic and elemental carbon fractions will help elucidate the contribution of diesel and gasoline combustion to the total ECAT.

We were also limited in the ability to distinguish the effects of exposure to ECAT during specific time periods of life due to the high degree of correlation between ECAT exposure throughout life. It is possible, given the correlation between estimated ECAT exposure throughout the first 3 years of life (Table 1), that the observed effects are associated with current exposure. Furthermore, the children enrolled in the CCAAPS cohort are at-risk children (i.e., born to at least one atopic parent). Therefore, the results of this analysis may not be generalizable to children born to nonatopic parents.

In conclusion, this study demonstrates that children exposed to traffic-related particles before age 12 months are at increased risk for the development of persistent allergic wheeze at age 36 months. The effect of high traffic-related particle exposure is accentuated in children coexposed to high levels of endotoxin. These findings support the hypothesis of synergistic interactions between immune function development and potential damage to the infant's developing lung resulting in early-onset persistent allergic wheeze. Airway inflammation and remodeling have been suggested as underlying mechanisms of asthma (50) and exposure to DEP and endotoxin, both separately and concurrently, results in the production of ROS and airway inflammation. Early persistent wheezing is a distinct wheezing phenotype and a significant risk factor for the development of asthma at later ages (51). Follow-up of this cohort will confirm the asthma phenotype with objective measures, including pulmonary function testing, and the effects of early environmental exposures.

***Conflict of Interest Statement***: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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