

Importance of the personal endotoxin cloud in school-age children with asthma

Nathan Rabinovitch, MD,^a Andrew H. Liu, MD,^a Lening Zhang, MSc,^b
Charles E. Rodes, PhD,^c Karin Foarde, PhD,^c Steven J. Dutton, MSc,^d
James R. Murphy, PhD,^b and Erwin W. Gelfand, MD^a *Denver, Colo, and*
Research Triangle Park, NC

Background: A number of studies have observed associations between the amount of endotoxin in urban dust and chronic asthma severity, but a direct relationship between personal exposure to household endotoxin and acute asthma worsening has not yet been defined.

Objective: We sought to investigate the relationship between day-to-day changes in personal endotoxin exposure and asthma severity.

Methods: In the winter and spring of 1999 through 2000, endotoxin exposures were monitored in asthmatic schoolchildren by using portable, as opposed to stationary, monitors designed to measure inhalable and respirable particulate matter less than or equal to 2.5 and 10 μm in diameter. Children were followed with daily measurements of FEV₁ and asthma symptoms.

Results: Over a 24-hour period, median daily personal endotoxin exposures ranged from 0.08 EU/m³ (measured at a particulate matter size range $\leq 2.5 \mu\text{m}$ in diameter) to 0.37 EU/m³ (measured at a particulate matter size range $\leq 10 \mu\text{m}$ in diameter). Personal exposures were significantly ($P < .001$) higher than endotoxin measurements from either indoor or outdoor stationary monitors. Moreover, individual exposures did not correlate with stationary measurements, suggesting that exposures derived from sources in close proximity to the children's personal activities might be better correlated with disease severity. Increases in personal endotoxin exposures were associated with decreased FEV₁ values and increased symptoms.

Conclusions: These findings demonstrate the importance of using personal monitoring to both measure and correlate endotoxin exposure with asthma severity. (*J Allergy Clin Immunol* 2005;116:1053-7.)

Key words: Endotoxin, asthma, children, personal monitoring

Exposure to endotoxin has been linked to occupational and other lung diseases.¹⁻³ Endotoxin inhalation in challenge

Abbreviations used

IQR: Interquartile range

PM_{2.5}: Particulate matter $\leq 2.5 \mu\text{m}$ in diameter

PM₁₀: Particulate matter $\leq 10 \mu\text{m}$ in diameter

RTI: Research Triangle Institute International

settings can induce the hallmarks of asthma: bronchoconstriction, airways inflammation, and bronchial hyperresponsiveness.^{4,5} Residential house dust endotoxin levels have been correlated with asthma symptoms and disease severity⁶⁻⁹ on the one hand, whereas other studies have suggested that exposure to microbial factors, including endotoxin, in early childhood might protect individuals against the development of an atopic state.^{10,11}

One limitation in many of these studies associating the effects of endotoxin on health outcomes is the absence of precise measurements of breathing zone exposure. The majority of studies on household endotoxin rely on levels determined in vacuumed dust. However, dust endotoxin is poorly correlated with airborne endotoxin levels.¹² Additionally, dust sampling does not allow for the assessment of daily exposure and its relationship to asthma control. Such surrogate exposure measurements complicate attempts to develop an accurate assessment of the relationship between specific levels of endotoxin exposure and asthma worsening.

Personal monitoring has generally been considered to be impractical and to pose an excessive burden, especially in school-age children. However, newly developed, small portable monitors that can measure respirable endotoxin exposure might be more acceptable than previous models. Using this technology, we examined the relationship between personal endotoxin exposure and health effects: quantifying levels of endotoxin that asthmatic children are exposed to on a daily basis, examining how well stationary monitors estimate endotoxin exposures, and determining whether a direct association between acute endotoxin exposure and disease severity in children with asthma could be documented.

METHODS

Study panels

Subjects were schoolchildren aged 6 to 13 years who attended the Kunsberg School at the National Jewish Medical and Research

From ^athe Department of Pediatrics, ^bthe Division of Biostatistics, and ^dthe Department of Occupational Medicine, National Jewish Medical and Research Center, Denver, and ^cResearch Triangle Institute International, Research Triangle Park.

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Reprint requests: Nathan Rabinovitch, MD, National Jewish Medical and Research Center, 1400 Jackson St, Denver, CO 80206. E-mail: rabinovitchn@njc.org.

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TABLE I. Particulate exposure–monitoring study design

Interval	No. of children sampled	Interval dates	Sampling period	Type of sample	No. of valid samples*
1	10	January 24–February 24, 2000	Ten consecutive schooldays	PM _{2.5} Personal Indoor Outdoor	84 Personal 19 Outdoor 16 Indoor
2	14	April 3–May 25, 2000	Two consecutive schooldays repeated 3 times†	PM ₁₀ Personal	80 Personal

*See Methods section for sample inclusion and exclusion criteria.

†Three pairs of days equally spaced throughout the interval.

**FIG 1.** Child wearing the personal monitor.

Center and had physician-diagnosed asthma. The Kunsberg School is a public elementary school designed to address the educational needs of children with significant asthma that interferes with regular school attendance and progress. This school was designed to minimize exposures that might trigger asthma.

Ethical and scientific approval for this study was obtained from the National Jewish and Research Triangle Institute International Institutional Review Boards before initiation of recruitment.

Particulate exposure–monitoring study design

Data on the particulate exposure–monitoring study design are shown in Table I. Personal particulate air sampling was accomplished in collaboration with Research Triangle Institute International (RTI, Research Triangle Park, NC). Particles were collected on 37-mm Teflon filters (Gelman Teflo; Pall Gelman Labs, Ann Arbor, MI) with an RTI version of the MSP Corporation's (Minneapolis, Minn) personal exposure monitor inlet.¹³ The sampling system ran at a flow rate of 2 L/min, weighed less than 2 lbs, and was designed to be worn as a waist pack or as a small backpack (Fig 1). System components could also be configured to sample particle concentrations at fixed locations indoors and outdoors for comparison. In addition, motion was recorded to quantify the subject's activity level. The system can collect particulate matter equal to or less than 10 μ m in diameter (PM₁₀; the size range that can penetrate into the larger airways) or equal to or less than 2.5 μ m in diameter (PM_{2.5}; the size range that can penetrate into the bronchoalveolar airways) but not both simultaneously.

The first part of the study (interval 1) was performed during a 1-month period in January and February, and the second part (interval 2) was performed over a 2-month period in April and May 2000. During interval 1, 10 children divided into 2 groups of 5 (because of the limited equipment available) participated in sampling over 10 consecutive schooldays. During interval 2 (April–May), sampling was performed in 14 children over the 2-month period. Because of the limited hardware available during this interval, children were divided into 4 groups. Six samples were obtained from each subject in 3 pairs of consecutive days spaced evenly throughout this period to obtain sampling for each child throughout the study.

Filters were changed at the beginning of each school day (8 AM–8 AM) to maximize the mass loading per sample. Thus each filter collected represented a 24-hour sampling period. In interval 1, PM_{2.5} monitoring was performed, whereas PM₁₀ was monitored in interval 2. In interval 1, stationary monitoring was also performed inside and outside the school in a fixed location and concurrent with personal monitoring with the same type of monitor. The indoor monitor was located in a main hallway at the center of the school 1.5 m from the floor. The outdoor monitor was located in a specially designed outdoor enclosure located on the roof of the single-story school away from any rooftop obstruction.

Subjects were instructed to wear the monitor during waking hours and to remove it and place it by their bed during sleep. Compliance with personal sampling was maximized by close monitoring of children inside the school, the use of the activity sensor, and an incentive program to increase compliance at home.

Baseline and daily exposure-related questionnaires

Families completed a questionnaire before the study began describing demographic characteristics and indicators of asthma severity.

Pulmonary function

All health outcomes were monitored daily throughout the study period. The primary health outcome in this study was FEV₁. Each child was asked to perform forced expiratory maneuvers with an Airwatch electronic asthma monitor (Carlsbad, Calif). These maneuvers were performed at the beginning of each school day (7:30–9 AM) and then repeated at home in the evening (5–9 PM). The morning FEV₁ maneuver was performed immediately after the 24-hour personal monitoring period, and the evening FEV₁ maneuver was performed 9 to 12 hours after the monitoring interval.

Symptoms

Children completed asthma symptom score diaries on arrival at school each morning (immediately after removing the monitor) and in

TABLE II. Demographics (from baseline questionnaire)

Interval	Male sex	Mean age, y (SD)	African American	Median prednisone bursts* (25 th -75 th IQR)	Albuterol, puffs per day (SD)	Symptoms, days per week (SD)
1	7 (70%)	8.8 (1.0)	7 (70%)	3.5 (11.0)	6.7 (2.6)	2.3 (2.3)
2	7 (50%)	9.0 (1.1)	11 (78.6%)	2.5 (3.0)	3.9 (2.1)	2.6 (2.6)

*In the year before the study.

the evening. The 5-point (0-4) asthma symptom score was based on the severity of asthma symptoms, with 0 representing no symptoms and 4 representing symptoms severe enough to prevent play or sleep.

Bronchodilator use

Each child was given 2 Dosers (Meditrak, Hudson, Miss), one for use at school and one for home. The Doser is an electronic counter that recorded the number of bronchodilator (albuterol) activations in each 24-hour period. In addition, albuterol nebulizer treatments were recorded on diary cards once daily. The values for nebulizer use were combined with the Doser data to produce one daily composite value for medication use.

Endotoxin measurements

Filters were weighed to quantify particulate mass before and after each monitoring interval, according to the procedure of Lawless and Rodes.¹⁴ Endotoxin measurements for interval 1 was performed at RTI. Measurements for interval 2 samples were performed at National Jewish, as previously described, with slight modifications.^{15,16} Briefly, endotoxin was eluted from the Teflon filters in 10 mL of sterile, pyrogen-free water. The eluate was then assayed with the Limulus Amebocyte Lysate kit, a US Food and Drug Administration–standardized bioassay for endotoxin (QCL-1000; Bio-Whittaker, Walkersville, Md). Intrasample laboratory variability with this approach was less than 5%. Negative control Teflon filters were assayed concurrently, along with a positive control, *Escherichia coli*–derived LPS samples with known endotoxin levels, including low-level concentrations (ie, 1, 10, or 50 pg/mL). Results for the negative control (field blank) Teflon filters revealed a very low level of background endotoxin (mean, 0.0018 EU/m³).

Statistical analysis

The SAS statistical analysis package (version 8.2; SAS, Cary, NC) was used for all analyses. A mixed model analysis was used, with a spatial exponential covariance structure to account for within-subject serial correlation. FEV₁ was analyzed as a continuous variable with the SAS procedure PROC MIXED. For analysis of medication use and symptom scores, the SAS GLIMMIX macro was used where a spatial exponential covariance structure was specified.

RESULTS

Demographics

Table II shows the demographic and asthma severity characteristics of the groups on the basis of a questionnaire administered to parents before the beginning of the study.

Compliance

Using the movement-activity logger, we estimated that the personal monitors were worn for a mean of 85% (interval 1) and 93% (interval 2) of the waking hours. Samples were omitted if the sampler flow rate deviated by more than 10% from the 2 L/min set point or if visible

debris ended up on the filter surface. Only filters worn for at least 75% of the waking hours were used in the analyses to obtain a true measure of personal exposure. As a result, a total of 6 and 11 filters were removed from the analysis for intervals 1 and 2, respectively.

Personal exposures versus stationary measurements

The median personal (PM_{2.5}) endotoxin exposure level in interval 1 was 0.08 EU/m³ (interquartile range [IQR], 0.09 EU/m³). In interval 2 the median (PM₁₀) endotoxin level was 0.37 EU/m³ (IQR, 0.16 EU/m³). In interval 1 personal exposures were significantly higher than stationary (indoor and outdoor school) levels of endotoxin (geometric mean, 0.07 EU/m³ [SD, 3.74 EU/m³] vs 0.02 EU/m³ [SD, 1.75 EU/m³], $P < .0001$; Fig 2). There was a wide variation in daily endotoxin exposures for many of the subjects. Variability in personal endotoxin measurements was not correlated with ambient levels ($r = 0.08$, $P = .75$) or levels inside the school ($r = 0.32$, $P = .17$), as measured with the stationary monitors.

Relationship between asthma severity and endotoxin exposure

During interval 2, the mean morning FEV₁ and evening FEV₁ values were 1.57l and 1.48l respectively (SD, 0.62l). Median bronchodilator use was 2.0 puffs per day (IQR, 5 puffs per day), and children complained of symptoms on 16% of days. Immediately after the monitoring period, children were significantly more likely to complain of asthma symptoms related to sleep if they had been exposed to higher levels of endotoxin (odds ratio, 2.042 per 1 EU/m³ change in personal endotoxin exposure; 95% CI, 1.029-4.042; $P = .041$). Increases in symptoms related to play were not significant (odds ratio, 1.575 per 1 EU/m³ change in personal endotoxin exposure; 95% CI, -0.723 to 3.428; $P = .248$). FEV₁ levels controlled for albuterol use measured in the morning immediately after monitoring were not significantly decreased (-107 mL per 1 EU/m³ change in personal endotoxin exposure; 95% CI, -312 to 99; $P = .306$). Evening FEV₁ levels measured 9 to 12 hours after the end of the monitoring period were significantly lower if children were exposed to higher levels of endotoxin (-316 mL per 1 EU/m³ change in personal endotoxin exposure; 95% CI, -597 to -36; $P = .036$).

Similarly, during interval 1, evening FEV₁ decreases trended ($P = .15$) lower in association with personal endotoxin exposures. This decreasing trend was not observed

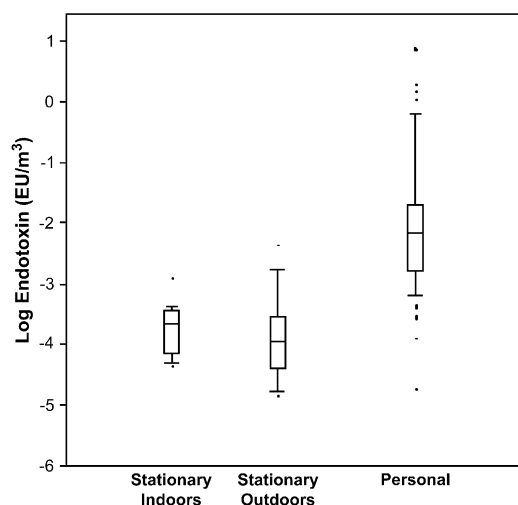


FIG 2. Personal exposures are higher than predicted by stationary monitors. The mean personal exposures compared with those measured by using stationary monitors running concurrently inside and outside the school are shown.

with area-wide endotoxin concentrations measured inside the school ($P = .80$, Fig 3).

DISCUSSION

This study is the first to successfully quantify personal endotoxin exposure in children and demonstrates a correlation between increases in natural endotoxin exposure and daily asthma control. Although this study was limited by the number of participants, it demonstrates the applicability and importance of personal monitoring in assessing the effects of endotoxin exposure in children with asthma. During interval 1, personal exposures were compared with the concentrations measured by using stationary monitors. This emphasized that concentrations measured with surrogate monitors are poorly correlated with true exposures and might not allow for an accurate assessment of the relationship between specific levels of endotoxin exposure and asthma worsening. Data collected during interval 2 demonstrated that endotoxin exposures were indeed associated with an individual's day-to-day asthma control.

Assuming that school-age children in this study have a typical minute ventilation of approximately 4 L/min at rest,¹⁷ the median amount of endotoxin inhaled over 24 hours approximates 2.2 EU/d or 220 pg/d for PM₁₀ exposures and approximately 0.46 EU/d or 46 pg/d for PM_{2.5} exposures. However, exposures can vary greatly within the same subject, depending on daily activities.

An important observation in this study was that personal exposures to endotoxin were significantly higher than area-wide measurements with a stationary monitor. This suggests that endotoxin exposures are not spatially homogenous but are derived from sources close to the child, forming a "personal cloud" similar to the one described for particulates.¹⁸ This spatial heterogeneity is also suggested by previous studies that observed considerable variability in dust endotoxin levels within individual

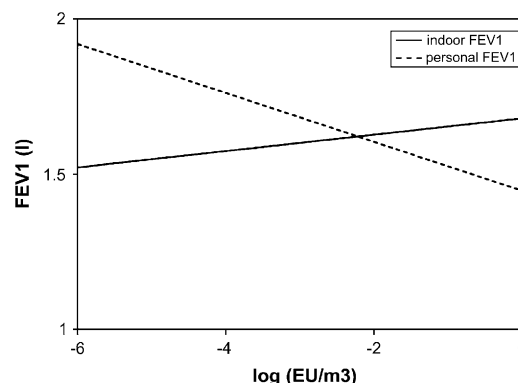


FIG 3. Relationship between endotoxin and FEV₁ as measured with the personal versus indoor stationary monitor. Shown are the regression of personal (broken line) and stationary indoor (solid line) log endotoxin measurements on evening FEV₁.

households¹² and office buildings.¹⁹ Thus exclusive use of indoor dust or even stationary indoor airborne monitors would provide poor estimates of exposure, and personal exposure monitoring might be essential to avoid exposure misclassification.

Previous studies have observed relationships between asthma severity and chronic exposure to endotoxin. These studies have been limited by between-subject factors that covary with household levels of endotoxins. Personal monitoring allows examination of this association by using a time-series approach. This can be helpful because it sets each individual to serve as his or her own control subject, limiting confounding caused by between-subject variables or time-dependent variables not related to endotoxin exposure. It also enables determination of the temporal nature of effects. Although the power of this study was limited by the number of subjects, we nonetheless observed significant associations between endotoxin exposure and changes in asthma severity, including decreases in lung function. The magnitude of the FEV₁ decrease was clinically significant, with estimates of 100 mL/min per interquartile change in endotoxin exposure. Additionally, endotoxin levels were significantly associated with increased symptoms, further confirming that endotoxin effects were clinically significant. Although not statistically significant (possibly because of decreased variability in measuring endotoxin in PM_{2.5} instead of PM₁₀ and the smaller number of individuals monitored), similar trends were observed in interval 1 by using personal exposures but not indoor endotoxin concentrations.

The effects on lung function and symptoms observed in this study are similar to the timing of effects observed in challenge settings, in which significant FEV₁ decreases were observed within 1 to 8 hours after a brief exposure challenge²⁰⁻²² and returned to baseline 24 hours after the challenge.²⁰ In these settings endotoxin levels required to induce changes in asthmatic subjects were considerably higher (20 µg) than exposures observed in this study.^{4,21,22} Other studies have calculated thresholds as low as 9 ng/m³ in healthy adults.²⁰ We suggest that children are exposed to repeated and brief but substantial

endotoxin exposures with certain household activities that contribute to the endotoxin personal cloud. Although 24-hour integrated exposures appear lower, these exposure spikes might in fact be similar to doses received in manipulated challenge settings. Additionally, endotoxin in the natural setting might become more potent with different coexposures and subject variables, including genetic determinants⁵ and underlying disease state.²³

In summary, daily endotoxin exposure in asthmatic children was measured in both PM_{2.5} and PM₁₀ particulate fractions. Personal endotoxin exposures were higher than area-wide measurements with stationary monitors, suggesting a significant personal cloud detected only with personal monitors. Increases in asthma severity indices were related to naturally occurring endotoxin exposures at clinically significant levels. These results highlight the importance of personal monitoring of endotoxin levels to better define the relationships between endotoxin exposures and asthma in children.

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