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Domestic exposure to endotoxin and respiratory morbidity in former smokers with COPD

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Abstract

Indoor air pollution has been linked to adverse COPD health, but specific causative agents have not yet been identified. We evaluated the role of indoor endotoxin exposure upon respiratory health in former smokers with COPD. Eighty-four adults with moderate to severe COPD were followed longitudinally and indoor air and dust samples collected at baseline, 3 and 6 months. Respiratory outcomes were repeatedly assessed at each time point. The associations between endotoxin exposure in air and settled dust and health outcomes were explored using generalizing estimating equations in multivariate models accounting for confounders. Dust endotoxin concentrations in the main living area were highest in spring and lowest in fall, while airborne endotoxins remained steady across seasons. Airborne and dust endotoxin concentrations were weakly correlated with one another (r_s=+0.24, p = 0.005). Endotoxin concentrations were not significantly associated with respiratory symptoms, rescue medication use, quality of life, or severe exacerbations. In-vitro whole blood assays of the pro-inflammatory capacity of PM_{10} filters with and without endotoxin depletion demonstrated that the endotoxin component of indoor air pollution was not the primary trigger for IL-1β release Our findings support that endotoxin is not the major driver in the adverse effects of indoor PM upon COPD morbidity.

Keywords

Endotoxin; Indoor air; COPD; IL-1 β; Particulate matter; Pollution

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INTRODUCTION

Chronic obstructive pulmonary disease (COPD), the fifth leading cause of death in the world, (World Health Organization 2015) commonly originates from toxic environmental exposures such as airborne pollutants. In developed regions, tobacco smoke is the primary culprit and results in a progressive disease characterized by lung injury and inflammation. However, even long after smoking cessation has been achieved, there is evidence for ongoing airway inflammation and persistent deterioration of lung function (Hogg 2006; Willemse et al. 2005). This observation has implied that continued exposure to other environmental factors might play a role in perpetuating the disease process.

Our group previously published work demonstrating that COPD morbidity among former smokers was influenced by environmental insults, specifically indoor air pollution. Exposure to fine particulate matter measuring less than 2.5 μm ($PM_{2.5}$) within the home, even at relatively low levels, was positively associated with higher respiratory morbidity, including increased symptoms, medication use, and risk of exacerbations (Hansel et al. 2013). However, airborne particulate matter is known to be a composite mixture of a variety of solid and liquid compounds, and it is still unclear which of the many components of $PM_{2.5}$ may be driving these observed adverse effects.

Of the biological compounds present in $PM_{2.5}$, endotoxin, a lipopolysaccharide (LPS) present in the outer membrane of Gram-negative bacteria that is ubiquitous in the environment (including as a component of tobacco smoke (Hasday et al. 1999)) is already established as a pro-inflammatory agent. The destructive effects of endotoxin to the lung have been illustrated by mechanistic studies performed in animal models and human challenge studies, which found LPS to invoke inflammatory cascades including cytokine release and recruitment of leukocytic mediators (Nightingale et al. 1998; O'Grady et al. 2001; Singh and Schwartz 2005; Vernooy et al. 2002). This response has been shown to ultimately result in both morphologic and physiologic changes that mimic those in COPD (Hakansson et al. 2012; Korsgren et al. 2012). However, despite experimental evidence regarding the adverse health effects of endotoxin, less is known about the significance of household exposure among vulnerable populations such as individuals with COPD.

It is critical to determine if domestic endotoxin may be the root cause of the observed adverse respiratory health outcomes, as interventions to improve indoor air quality could preferentially target this specific component of indoor air pollution. Furthermore, airborne levels of endotoxin may be dynamically related to household reservoirs of settled dust that resuspend into the air during human activities, highlighting the importance of accounting for both sources in exposure assessments. Therefore, to determine whether in-home exposure to endotoxin may be driving the adverse respiratory effects of indoor PM, we investigated the impact of airborne and settled floor dust endotoxin in a longitudinal cohort of former smokers with COPD.

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MATERIALS AND METHODS

Participant Recruitment

Health and environmental data, including home endotoxin concentrations, from all 84 participants of the original cohort (Hansel et al. 2013) were analyzed for this study. Details of methods have been previously described (Hansel et al. 2013). Briefly, adult former smokers with COPD living in the Baltimore area were recruited. The study protocol was approved by the Johns Hopkins Institutional Review Board. Participants were provided a written consent and enrolled if they met the following inclusion criteria: 1. Age ≥ 40 years 2. post-bronchodilator FEV_1≤ 80% predicted 3. FEV_1/FVC <70% and 4. > 10 pack years smoking, with quit date >1 year prior to enrollment and confirmation of nonsmoking status by screening exhaled carbon monoxide level ≤ 6 ppm. Participants were excluded if they had other pulmonary disorders or alpha-1 antitrypsin deficiency, or had taken oral corticosteroids in the 3 months prior.

Endotoxin Assessment

Both airborne and settled floor dust levels of endotoxin were assessed in the home. Household indoor airborne sampling for $PM_{2.5}$, PM with an aerodynamic size 2.5-10 μm ($PM_{2.5-10}$, i.e. coarse PM), and PM_{10} and settled floor dust collection were performed in the participant's main living area over one-week periods at baseline, 3, and 6 months, in a manner described previously (McCormack et al. 2009). Settled dust was collected using a clean vacuum nozzle and a new collection sleeve (Mitest®). A floor surface encompassing a 200cm × 100cm area was vacuumed approximately for a 4-minute period. Settled floor dust sampling was performed after air sampling was completed in order to prevent contamination from dust resuspension related to floor vacuum sampling.

Air filters and ten milligrams of each sample of main living area settled floor dust samples were extracted for endotoxin analysis in sterile, pyrogen-free water (Thermo Scientific, Waltham MA USA) containing 0.05% Tween-20 for 1 hr at 22°C with continuous shaking as previously described (Spaan et al. 2008). Extracts were centrifuged and supernatants were transferred into pyrogen-free cryotubes. The cryovials were stored at −80°C until analyzed for endotoxin and whole blood pyrogen assays.

Settled dust was also collected from the bedroom floor using similar methods, and for samples that had insufficient dust collected from the main living area (n=42, 18%), bedroom floor settled dust samples were included, if available.

Endotoxin concentration of airborne (using PM_{10} filters) and dust samples was determined with the Pyrochrome® kinetic Limulus amoebocyte lysate assay (LAL) following manufacturer's instructions (Associates of Cape Cod Inc, East Falmouth, MA USA). The concentration of endotoxin in the filter extracts was determined by preparing a standard curve (0.005 to 5 endotoxin units [EU]/mL) with standards from *E. coli* O113:H10 (Associates of Cape Code) prepared in pyrogen-free water with 0.05% Tween 20. The limit of detection (LOD) was determined to be 0.005 EU/mL for airborne endotoxin and 0.0145 EU/mL for settled floor dust.

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Co-pollutant Assessment

Exposure to second hand smoke (SHS) was determined by indoor air nicotine concentrations at baseline only and considered to be present if any sample had detectable air nicotine (LOD 0.02 μg/m^3) (Hammond and Leaderer 1987). Hair nicotine was measured at all visits (LOD 0.025 ng/mg) (Butz et al. 2011). Indoor air concentrations of NO_2 were measured at each monitoring period (baseline, 3 and 6 months) using a passive sampler (Ogawa badge) (Palmes et al. 1976), with an LOD of 0.52 ppb.

Clinical Evaluation

Participants were evaluated at three clinic visits occurring during each 1 week long in-home air quality assessment performed at baseline, 3 and 6 months. Initial spirometry, before and after albuterol, was performed at the clinic visit according to American Thoracic Society (ATS) guidelines (Hankinson et al.1999; Miller et al. 2005). Baseline respiratory symptoms were assessed through validated questionnaires, including dyspnea (Medical Research Council (MRC) dyspnea scale (Bestall et al. 1999), respiratory health (modified ATS-DLD), (Ferris 1978) and quality of life (St. George's Respiratory Questionnaire (Barr et al. 2000). The frequencies of cough, sputum, or wheezing within the last 4 weeks were all categorized as one of the following responses: “almost every day, several days a week, a few days a month, only with respiratory infections, or not at all.” Nighttime symptoms were considered dichotomously as “yes” or “no” responses to coughing or breathing that disturbed sleep. Daily symptom diaries captured frequency of rescue medication use (0, 1, 2, 3, 4, or >4 times per day) as well as concurrent respiratory symptoms assessed by the Breathlessness, Cough, and Sputum Scale©, or BCSS, (Leidy et al. 2003), and responses averaged over each one-week monitoring period. COPD exacerbation events, defined as worsening symptoms requiring antibiotics, oral steroids, or an acute healthcare encounter (severe exacerbation if respiratory symptoms resulted in an emergency room visit or hospitalization), were assessed during each clinic visit and by monthly telephone calls.

Whole Blood Pyrogen Assay

A human-based *in-vitro* method, known as the human whole blood pyrogen assay (Hartung and Wendel 1995), was used to further investigate the pro-inflammatory potency of indoor air PM in a subset (n=26) of PM_{10} samples, which were randomly selected with the random (=RAND) and index (=INDEX) functions in Excel™ (Microsoft Corp., Redmon, WA). There were no statistical significant differences in PM concentrations or housing characteristics between those included in the current analyses and the overall study (data not shown).

Cryopreservation of blood—With the approval of the Johns Hopkins Bloomberg School of Public Health (JHSPH) Institutional Review Board, twenty-five healthy subjects from staff of the JHSPH were recruited and 30 mL of peripheral blood collected from each volunteer by venipuncture into sterile heparin tubes (Becton, Dickinson & Co.). The blood samples from all 25 volunteers were pooled to use in the assay in order to avoid variability of pro-inflammatory cytokine induction across individual samples. The tubes were placed in

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ice water before cryopreserving the collected blood as previously described. (Schindler et al. 2004)

Assessment of the pro-inflammatory potency of PM_{10} samples—The human whole-blood pyrogen assay was performed in pyrogen-free tissue culture microplates with whole extracts and endotoxin-depleted extracts (Daneshian, von Aulock, Hartung 2009). The concentration of endotoxin in the extract prior to depletion was determined by the LAL assay and subsequently expressed relative to the total mass of PM on the filter. Whole blood incubations were completed in sterile microplates, which were prepared with the extract from filter samples, as well as endotoxin-depleted extracts. For the incubation of extracts, 20 μL of extract and 200 μL (170 μL in case of endotoxin-depleted extracts) of RPMI 1640 (Life Technologies, Grand Island, NY USA) were added to wells of microplates. To deplete endotoxin from the extracts, 25 IU of polymyxin B sulfate (Xgen pharmaceuticals, Big Flats, NY) (Thomas et al. 1998) was added to each of the correspondent wells and incubated with the extracts at 37 °C. Because in preliminary experiments, 25 IU of polymyxin B demonstrated the ability to neutralize the pro-inflammatory potency of up to 5 EU of lipopolysaccharide without compromising the reactivity of the immune cells in the whole blood (data not shown), those extracts with higher levels of endotoxin were reduced to levels ≤5 EU prior to the assay in order to achieve full depletion by polymyxin B in each well). After 1 hr, 20 μl of thawed cryopreserved pooled blood (used within 15 minutes after being thawed at 37 °C) was added to the wells and the microplates incubated for 18 h at 37 °C, 5% CO_2. The total volume per/well was 240 μl. A positive endotoxin control (5 EU of lipopolysaccharide from *E. coli* O113:H10, Associates of Cape Cod) and negative control (RPMI 1640) were handled in parallel to the samples. At the end of the incubation period, the concentration of interleukin (IL)-1β, a known pro-inflammatory cytokine linked to COPD (Chung and Adcock 2008; Xiao et al. 2014) in the supernatants was determined by ELISA (R&D Systems, Minneapolis, MN USA). The LOD for IL-1β was 7.8 pg/mL.

Statistical Analysis

Descriptive analyses were performed using means and proportions, T-tests, Spearman correlations, nonparametric one-way analysis of variance (ANOVA), as appropriate. At each time point, in-home endotoxin concentrations were log transformed (base 10) and separately evaluated as exposure variables in generalized estimating equation (GEE) models (Diggle et al. 2002), in order to account for repeated measures of the outcomes over time. Analyses were adjusted for age, sex, education, season, and baseline pre-bronchodilator percent predicted FEV_1. In primary analyses of settled dust endotoxin, the room of settled dust collection (main living area or bedroom floors) was included as a confounder variable. Sensitivity analyses of settled dust endotoxin were conducted including only main living room floor samples and excluding samples that were collected from the bedroom floor. To evaluate the effect of pollutants on respiratory health, continuous outcomes and binary outcomes (i.e. nocturnal symptoms, exacerbations) were analyzed using negative binomial and logistic regression models, respectively, with endotoxin concentrations considered as continuous predictors. Air endotoxin analyses were also performed with models including $PM_{2.5}$ as a covariate, as $PM_{2.5}$ was previously shown to be associated with COPD outcomes (Hansel et al. 2013). We also tested for interaction between endotoxin concentrations and

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other indoor air pollutants, including air nicotine and nitrogen dioxide (NO_2). All analyses were performed with StataSE software, version 12.0 (Stata Corp, College Station, TX) and a P value less than 0.05 was considered statistically significant.

For the whole blood pyrogen assay, a paired T-test was performed to determine significant differences in the concentration of induced Il-1β between whole extracts and endotoxin-depleted extracts. Statistical analyses were performed with SPSS V21 0.0.0 64-bit (IBM, Armonk NY).

RESULTS

Participant Characteristics

Demographic and health characteristics of the eighty-four participants in this published cohort are as published previously (Hansel et al. 2013). Of note, the mean age was 69 years, with the majority being male (58%), and of predominantly Caucasian race (88%). **(Table 1)** Participants all had moderate or severe COPD, with mean (SD) pre-bronchodilator FEV_1 percent predicted values of 48.6 (16%). Participants reported spending 80% of the day within their own home. All were former smokers who had a mean (±SD) cigarette smoking history of 56.8 (±28.7) pack years, and had quit an average of 13 years prior.

Respiratory Health

At baseline, a significant number of participants had chronic respiratory symptoms, with 62% reporting cough, 48% sputum, 26% nighttime awakenings from COPD, and 18% wheeze, occurring several days a week. At baseline, the mean (±SD) MRC dyspnea score was 2.6 (±1.1), and mean (±SD) quality of life scores assessed by the SGRQ was 39.7 (±17.8). During the 6-month enrollment, 43% of participants reported exacerbations, 39 of which were moderate exacerbations and 16 severe exacerbations.

Pollutant Concentrations

Airborne endotoxin—The mean (±SD) airborne endotoxin concentration was 0.55 (±1.3) EU/m^3 [median 0.06 EU/m^3, IQR (0.01-0.36 EU/m^3]. Levels of airborne endotoxin were relatively constant across most seasons (median (IQR): spring 0.06 (0.02-0.26) EU/m^3; summer 0.11 (0.01-0.78) EU/m^3; fall 0.06 (0.02-0.25) EU/m^3; and winter 0.05 (0.003-0.30) EU/m^3, (p=0.46)), though there was a non-significant trend towards summer concentrations being higher than the rest of the year.

Settled floor dust endotoxin—The majority of dust was collected from a carpeted surface (95%). When analyzed separately, dust endotoxin collected from bedroom floors at visits where living area samples were insufficient (n=36) had a median concentration that was significantly lower than main living area values (74.3 (IQR 40.7, 129) EU/mg vs. 133.5 (86.3-236) EU/mg respectively, p=0.004). Endotoxin concentrations in living area settled floor dust varied slightly across seasons, with levels highest in the spring (median (IQR) 160 (122-236) EU/mg) and lowest in the fall (median (IQR) 104 (50.7-209) EU/mg, (p =0.03). Airborne endotoxin and settled floor dust concentrations in the main living area were weakly correlated (r_s=+0.24, p = 0.005) **(Figure 1)**.

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Other airborne pollutants—As reported previously, the median (±SD) $PM_{2.5}$ concentration in the main living area over the study period was 12.2 (±12.2) μg/m^3. (Hansel et al. 2013) Neither airborne nor dust endotoxin and $PM_{2.5}$ in the living area were significantly correlated (r_s=−0.02, p =0.78 and r_s = 0.16, p = 0.06, respectively). Similarly, neither airborne nor living room dust endotoxin levels, and $PM_{2.5-10}$, (a component of PM_{10}), were significantly correlated (r_s =0.138, p =0.09 and r_s = 0.2, p= 0.11, respectively).

Despite a minority (17%) of participants reporting a smoker living in the home, approximately half (54%) of the living areas had detectable air nicotine levels (mean (±SD) 0.16 (±0.42) μg/m^3 at baseline. NO_2 concentrations in the living area across all time points had a mean (±SD) of 12.2 (±11.8) ppb (median (IQR) 8.0 (5.2,16.1) and were not significantly correlated with air endotoxin levels (p=0.89).

Association Between Endotoxin Levels and COPD Morbidity

In bivariate analyses, increasing concentrations of air endotoxin were not associated with increased frequency of wheeze, rescue medication use, nocturnal symptoms, and worse quality of life (SGRQ). Similarly, there was not a statistically significant association between settled floor dust endotoxin and these respiratory health outcomes. After adjusting for confounders in multivariable analyses, neither airborne nor dust endotoxin was significantly associated with measures of COPD morbidity **(Table 2)**. In sensitivity analyses examining settled dust samples collected from the living room floor only and excluding bedroom samples, dust endotoxin was similarly not associated with respiratory outcomes (data not shown). In addition, despite the well-established effects of nicotine on respiratory health, and our recent published report of the independent effects of NO_2 on health in this cohort, neither the concentrations of indoor nicotine nor NO_2 modified the effect of endotoxin on respiratory health (data not shown). Lastly, including $PM_{2.5}$ into multivariate models of airborne endotoxin did not significantly impact the relationship of endotoxin to respiratory outcomes, and re-demonstrated the significant associations between $PM_{2.5}$ and respiratory outcomes that were reported in the initial analysis (data not shown) (Hansel et al. 2013).

Contribution of Endotoxin to Pro-inflammatory Reactivity of PM

The pro-inflammatory potential of the PM samples was determined based on the release of the pro-inflammatory cytokine IL-1β from human whole blood cells, after separately exposing immune cells in human whole blood to whole extracts and to endotoxin-depleted extracts. The mean (SD) endotoxin concentration in the whole extracts, prior to depletion, was 2.3 (3.4) EU/μg of PM. Whole extracts of PM filters induced a median IL-1β potency of 340 pg/mL (range 35 – 558 pg/mL) **(Figure 2)**. Endotoxin depletion resulted in a 23% decrease in the median of the IL-1β potency ($p < 0.005$). Endotoxin-depleted extracts samples still had a several fold higher IL-1β potency (median 263 pg/mL, range 11 – 517 pg/mL) than the negative control (7.8 pg/mL (range 6-9 pg/mL), demonstrating that endotoxin depletion did not fully abolish the inflammatory response of PM. Taken together, these data further indicate that a substantial amount of pro-inflammatory activity of PM is not modulated by endotoxin.

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DISCUSSION

Our analyses found that there was no significant association between airborne or settled floor dust endotoxin concentrations and respiratory health status in former smokers with COPD. We also demonstrated using a human whole-blood pyrogen assay that the presence of endotoxin in PM air samples did not explain the full pro-inflammatory potential of PM extracts, as measured by the induction of IL-1β, a cytokine which has been shown to play a role in the pathogenesis of chronic inflammation and airway destruction in COPD (Chung and Adcock 2008). These results suggest that endotoxin is an unlikely driving force behind the previously observed adverse health effects of PM in this population. A complete understanding of the impact of indoor pollutants upon respiratory health is essential because patients with COPD are known to spend the majority of time inside, often within their own homes (Leech and Smith-Doiron 2006), where specific environmental exposures may be responsible for the continued deterioration in lung health.

Overall, concentrations of endotoxin within house dust in Baltimore homes in this study (median 134 EU/mg) were similar to published reports in other urban communities, such as those found in New York City (75 EU/mg) (Perzanowski et al. 2006), as well as, specifically, COPD homes (96 EU/mg) (Osman et al. 2007). Consistent with national data (Thorne et al. 2009), concentrations of endotoxin in the dust derived from living areas were significantly higher than those collected from the bedroom, which may be reflective of the greater time spent by these participants within the living room, and has important implications for future exposure assessments of COPD homes. In addition, airborne endotoxin concentrations in our study (median 0.06 EU/m^3) were very close to other Baltimore homes (0.13 EU/m^3) (Mazique et al. 2011). However, we chose to report on the effects of both airborne as well as settled floor dust endotoxin, since each is known to have different degrees of variability and may impose their own distinct patterns of exposure upon the respiratory system. Acute inhalational exposure of endotoxin may be proportionate to immediate airborne concentrations generated by recent household activities. Alternatively, levels of endotoxin within settled floor dust may reflect more chronic long-term exposure within the home. Indeed, in this analysis, settled floor dust endotoxin levels and airborne endotoxin levels were only weakly correlated as also previously shown in other home environments (Mazique et al. 2011). This suggests that using one or the other exposure assessment could misestimate effects on lung health. However, given that we did not appreciate a significant impact of either settled or airborne endotoxin upon the burden of respiratory disease, we conclude that in-home endotoxin is not a primary agent within the pathway of pollution-induced morbidity in patients with COPD and that other putative components of particulate matter should be investigated.

Endotoxin is an appealing candidate to consider as the driving force behind PM's adverse health consequences. PM is known to contain a complex mixture of biologically derived components some of which, including cellular components of Gram-positive bacteria and fungi, have direct respiratory effects (Hulin et al. 2012). In particular, directly administered endotoxin in experimental settings has been demonstrated to induce destruction within the lung airways and parenchyma that simulate those present in COPD (Hakansson et al. 2012; Kobayashi et al. 2013; Korsgren et al. 2012). Occupational exposures to high levels of

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endotoxin, such as in farming environments, have also been linked to higher levels of airway inflammation and decreased lung function (Rylander 2006). Furthermore, bacterial infections are very often the trigger behind exacerbations of the disease; in fact, antibacterial agents are among the mainstays of effective therapy for these flares. It would be conceivable, therefore, that potent bacterial agents (i.e. endotoxin from Gram-negative bacteria) in the indoor environment would play a significant role in mediating the persistent inflammation within COPD lungs. Despite this attractive possibility, the theory that particulate matter may simply be a vehicle for an endotoxin component that provokes ongoing respiratory pathology is not supported by our results.

Our findings also highlight an important distinction between two entities of obstructive lung disease—asthma and COPD, as endotoxin exposure has been linked with outcomes in asthma and allergic disease (Liu 2004). A small number of studies have demonstrated that while exposure in the first year of life seem to be protective against allergic phenomena such as allergic sensitization and eczema (Braun-Fahrlander et al. 2002; Gehring et al. 2002; Phipatanakul et al. 2004), exposure to household endotoxin increases the risk of wheeze and asthma symptoms later in childhood (Celedon et al. 2007; Park et al. 2001; Ryan et al. 2009), and among those individuals already with asthma, a greater burden of disease (Michel et al. 1991; Michel et al. 1996; Rizzo et al. 1997). Consequently, though asthma and COPD may share similar airway pathophysiology, our work suggests that they have quite distinct relationships with regard to endotoxin exposure.

The lack of association in our study of endotoxin and COPD outcomes may have several explanations. One, it may reflect the true impotency of domestic exposure within this low range of endotoxin concentrations to effect clinically detectable changes in respiratory morbidity. Alternatively, the relationship between endotoxin and respiratory status may be modified by co-exposures to other pollutants. There is support of such a phenomenon in asthma related outcomes, such that in environments with either high air nicotine or low NO_2 concentrations, endotoxin was associated with worse asthma, whereas the opposite effect was seen in homes with undetectable nicotine and high NO_2 (Matsui et al. 2013). These findings highlight the overall complexity of airborne exposures with regard to effects on respiratory health. Though we are limited in power to determine effect modification, in order to tease out the effects of such co-pollutants within our COPD homes, we searched for interaction effects and found that neither air nicotine, nor NO_2, significantly modified the observed relationships in this cohort. Further analyses are needed to determine if there may be other co-pollutants within COPD homes that may have contributed to the negative effects reported here.

There are several limitations to this study. Endotoxin in this study was measured inside relatively cleaner Baltimore homes, which may not be generalizable to other types of homes or in other geographical regions. In addition, we did not specifically assess for personal exposure or outdoor pollutant levels, though the majority of time for each participant was spent in the home environment where endotoxin levels were measured, and as previously described, outdoor $PM_{2.5}$ concentrations did not contribute greatly to the variability of indoor concentrations (Hansel et al. 2013). Furthermore, while we had sufficient power in this cohort of 84 participants to previously show that $PM_{2.5}$ concentrations were linked to

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worse respiratory morbidity, it is possible that our sample size may have limited our ability to detect an association of health outcomes with endotoxin exposure. Additionally, because many of the houses were relatively clean, we were limited by a subset of the visits where house dust was insufficient to collect in the main living area (18%). However, results were similar when we included endotoxin concentrations from the bedroom in order to minimize missing data.

Of note, the airborne endotoxin assessed in this study was derived from filters that captured particles <10 μm, and therefore do not directly investigate endotoxin's role in $PM_{2.5}$'s effects upon COPD morbidity. While particles captured by our PM_{10} filters do also contain $PM_{2.5}$ and hence would include any associated endotoxin of smaller particles alike, we acknowledge that by analyzing endotoxin extracted from these more inclusive filters we cannot definitively exclude endotoxin's ability to mediate the health effects of $PM_{2.5}$. Likewise, we acknowledge that our assessment of endotoxin's pro-inflammatory effects was limited to IL-1β and future studies exploring the ability of endotoxin to induce other cytokines may be alternately revealing. Finally, while our participants were mostly older, Caucasian, former smokers with moderate or severe obstruction, it is known that COPD is a heterogeneous disease, whose wide range of subtypes and disease phenotypes are, as of yet, poorly understood and classified. Consequently, the impact of endotoxin in a mixed phenotypic population may be underestimated.

In one of the few studies of indoor pollutant exposure on COPD morbidity in a developed region, we report further analyses that demonstrate that endotoxin is likely not the main component of PM that is responsible for its adverse effects on COPD morbidity. Further investigations to identify the specific toxic components of indoor air pollution in this population are essential in developing novel environmental strategies to ameliorate respiratory morbidity and improve lung health in the face of limited therapeutic options.

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Practical Implications

Indoor environmental factors such as airborne particulate matter (PM) may worsen lung health, especially in people with pre-existing respiratory diseases who spend a significant time within the home. However, the specific components of PM responsible for adverse health effects are unknown, and therefore environmental strategies to protect the lung from these exposures have been limited. This research demonstrates that in former smokers with COPD, endotoxin--a bacterial component linked to airway inflammation--is likely not the main culprit behind the harmful effects of PM on the lung. Further understanding of harmful indoor environmental exposures and their adverse effects in the airway are key steps to designing non-pharmacologic interventions aimed at reducing environmental exposures that perpetuate illness in vulnerable patients.

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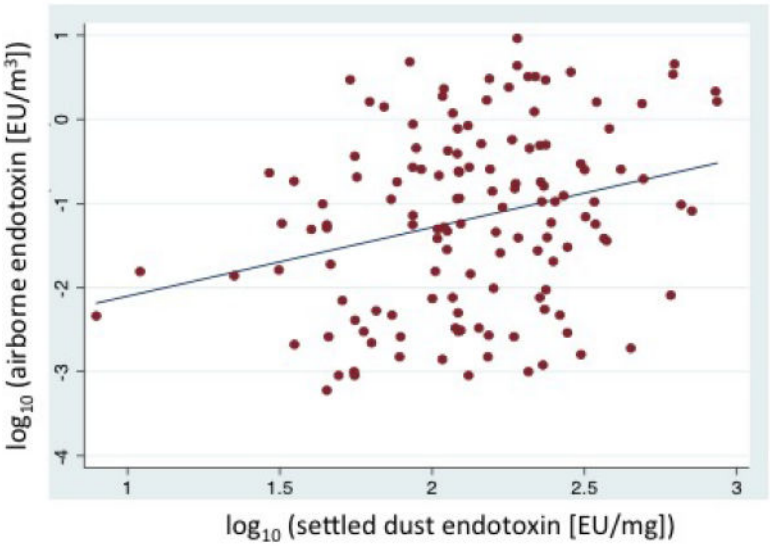

Figure 1.

Correlation between airborne and settled dust endotoxin concentrations. Concentrations of endotoxin in dust and air were weakly correlated (rs=+0.24, p = 0.005). X and Y scales are logarithmically transformed.

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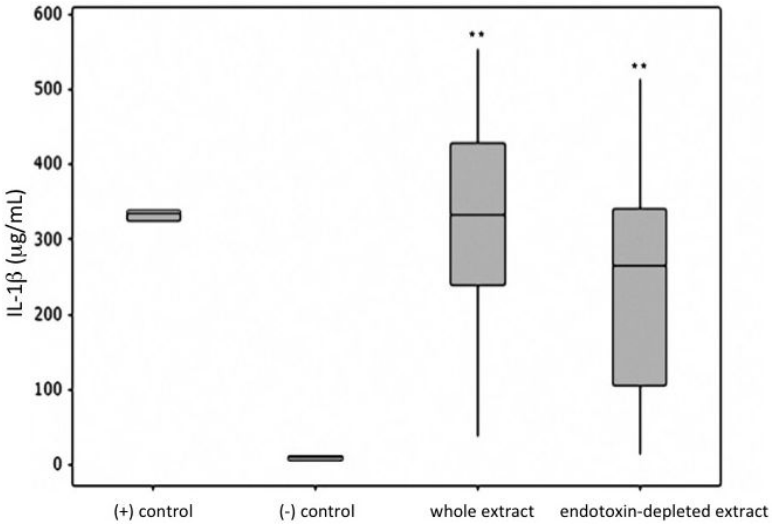

Figure 2.

Concentration of IL-1β induced in the whole blood pyrogen assay by whole extract and endotoxin-depleted extracts. Filter extracts, in the presence or absence of the endotoxin antagonist polymyxin B, and extracted filters were incubated in the thawed cryopreserved human whole blood and the concentration of induced pro-inflammatory cytokine IL-1β determined with ELISA. 5 EU from E.coli O113:H10 was the positive control. Pyrogen-free RPMI 1640 was the negative control. ** Statistical significant difference compared to the negative control. $p < 0.05$ was determined to be significant.

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Table 1

Participant Characteristics

Participant Characteristics	n=84
Age, mean years (SD)	68.9 (7.4)
Gender, n (%) male	49 (58)
Race, n (%)	
Caucasian:	74 (88)
Black/African American:	8 (10)
Other	2 (2)
Marital, n (%)	
Single:	8 (10)
Married:	39 (46)
Widowed:	21 (25)
Separated/Divorced	16 (19)
Education, n (%)	
< High School	17 (20)
High School	17 (20)
Some College	27 (32)
Bachelor's Degree	10 (12)
At least some Graduate School	13 (15)
Smoking History, mean (SD)	
Pack-Years	56.8 (28.7)
Years smoked	36.7 (10.7)
Last Cigarette (years since)	13.1 (9.1)
Secondhand smoke exposure, n (%)	
Reported smoking in the home	14 (17)
Presence of hair nicotine	21 (28)
Baseline Lung Function, mean (SD)	
Pre-BD FEV_1, L	1.35 (0.59)
Pre-BD FEV_1, % predicted	48.8 (15.9)
Pre-BD FEV_1/FVC, %	51 (10.5)
Post-BD FEV_1, L	1.46 (0.62)
Post-BD FEV_1, % predicted	52.8 (16.5)
Post-BD FEV_1/FVC, %	52 (10.5)
Baseline Health Status	
Gold Stage n (%)	
II	38(45)

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Participant Characteristics	n=84
III	35(42)
IV	11(13)
Baseline Health outcomes	
SGRQ, mean (SD)	39.7 (17.8)
MRC, mean (SD)	2.6 (1.1)
Nocturnal Symptom, n (%)	22 (26)
Usual Cough, n (%)	30 (36)
Usual Phlegm, n (%)	36 (43)
Exacerbations previous year, n (%)	16 (19)

Definition of abbreviations: BD= bronchodilator; GOLD=Global Initiative for Chronic Obstructive Lung Disease; MMRC=Modifed Medical Research Council; SD= Standard Deviation; SGRQ= St. George's Respiratory Questionnaire.

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Table 2

Multivariate analyses of the association between settled dust or airborne endotoxin concentrations with health outcomes in former smokers with COPD

	Per 1 unit increase in log_{10} airborne endotoxin [EU/m^3]			Per 1 unit increase in log_{10} settled dust endotoxin [EU/mg]		
	β	**p**	**95% CI**	**β**	**p**	**95% CI**
MMRC (dyspnea)	−0.03	0.66	(−0.15, 0.10)	0.18	0.33	(−0.18, 0.54)
Wheeze	0.08	0.37	(−0.10, 0.25)	−0.21	0.39	(−0.70, 0.27)
Frequency of Inhaler Use	−0.004	0.92	(−0.10, 0.87)	−0.015	0.93	(−0.34, 0.31)
SGRQ	0.17	0.80	(−1.14, 1.49)	1.62	0.50	(−3.07, 6.32)
	OR	**p**	**95% CI**	**OR**	**p**	**95% CI**
Nocturnal symptoms	1.06	0.74	(0.74, 1.52)	1.34	0.60	(0.45, 3.97)
Severe Exacerbations	0.66	0.20	(0.35, 1.25)	0.87	0.89	(0.12, 6.37)

Models include adjustment for age, gender, education, season of sampling, baseline pre-bronchodilator percent predicted FEV_1, and room of sampling (for settled dust models only)

OR: Odds ratio

MMRC: Modified Medical Research Council

SGRQ: St. George's Respiratory Questionnaire