Endotoxin in Concentrated Coarse and Fine Ambient Particles Induce Acute Systemic Inflammation in Controlled Human Exposures

Behrooz Behbod¹*, Bruce Urch²,⁵, Mary Speck², James A. Scott²,³, Ling Liu⁴, Raymond Poon⁴, Brent Coull¹, Joel Schwartz¹, Petros Koutrakis¹, Frances Silverman²,³,⁵,⁶,⁸, and Diane R Gold¹,⁷

¹Harvard School of Public Health, Boston, Massachusetts, USA
²Gage Occupational & Environmental Health Unit, St. Michael’s Hospital, University of Toronto, Toronto, Ontario, CANADA
³Division of Occupational and Environmental Health, Dalla Lana School of Public Health, University of Toronto, Toronto, Ontario, CANADA
⁴Health Canada, Ottawa, Ontario, CANADA
⁵Southern Ontario Centre for Atmospheric Aerosol Research (SOCAAR), Toronto, Ontario, CANADA
⁶Divisions of Occupational and Respiratory Medicine, Department of Medicine, University of Toronto, Toronto, Ontario, CANADA
⁷The Channing Laboratory, Brigham and Women’s Hospital, Harvard Medical School, Boston, Massachusetts, USA
⁸Keenan Research Centre, Li Ka Shing Knowledge Institute, St. Michael’s Hospital, Toronto, Ontario, CANADA

Abstract

Background—Knowledge of the inhalable particulate matter components responsible for health effects is important for developing targeted regulation.
Objectives—In a double-blind randomized cross-over trial of controlled human exposures to concentrated ambient particles (CAPs) and their endotoxin and (1→3)-ß-D-glucan components, we evaluated acute inflammatory responses.

Methods—Thirty-five healthy adults were exposed to five 130-minute exposures at rest: 1) fine CAPs (~250 μg/m³); 2) coarse CAPs (~200 μg/m³); 3) a second coarse CAPs (~200 μg/m³); 4) filtered air; and 5) medical air. Induced sputum cell counts were measured at screening and 24-hours post-exposure. Venous blood total leukocytes, neutrophils, interleukin-6 and high-sensitivity C-reactive protein (CRP) were measured pre-, 3 and 24-hours post-exposure.

Results—Relative to filtered air, an increase in blood leukocytes 24-hours (but not 3-hours) post-exposure was significantly associated with coarse [estimate = 0.44 × 10⁹ cells/L (95% CI: 0.01, 0.88); n=132] and fine CAPs [0.68 × 10⁹ cells/L (95% CI: 0.19, 1.17); n=132], but not medical air. Similar associations were found with neutrophil responses. An interquartile increase in endotoxin (5.4 ng/m³) was significantly associated with increased blood leukocytes 3-hours post-exposure [0.27 × 10⁹ cells/L (95% CI: 0.03, 0.51); n=98] and 24-hours post-exposure [0.37 × 10⁹ cells/L (95% CI: 0.12, 0.63); n=98]. This endotoxin effect did not differ by particle size. There were no associations with glucan concentrations or interleukin-6, CRP or sputum responses.

Conclusions—In healthy adults, controlled coarse and fine ambient particle exposures independently induced acute systemic inflammatory responses. Endotoxin contributes to the inflammatory role of particle air pollution.

Keywords
Endotoxin; Coarse Ambient Particles; Inflammation

INTRODUCTION

It is well recognized that exposures to inhalable ambient particles are associated with significant morbidity and mortality. Knowledge of the particulate matter components responsible for the health effects observed in epidemiological studies is of importance for the development of targeted air pollution regulations.

While fine (PM₂.₅) particles are being widely regulated, there is more uncertainty over the toxicity of coarse (PM₂.₅–10) particles. Fine particles, derived mainly from mobile and industrial emission sources, are associated with cardiovascular outcomes and are able to reach the alveolar region and deposit mainly by interception. Coarse particles have been shown to be associated with respiratory inflammation and disease as well as innate immune responses of airway macrophages, and tend to deposit by inertial impaction in the extrathoracic airways (nose and pharynx; above the vocal cords). Smaller coarse particles may also deposit onto the lower ciliated thoracic airways by gravitational sedimentation.

Gram-negative bacterial cell walls contain endotoxin (lipopolysaccharide; LPS), which is composed of polysaccharide chains, a connecting core, and a lipid A unit that is responsible for its toxic effects. Another ambient biological exposure that is highly correlated with endotoxin levels is (1→3)-ß-D-glucan (hereafter glucan). These compounds are glucose polymers that are non-allergic water-insoluble structural cell wall components of most
fungi, as well as some bacteria and plants. Their biological activity is independent of cell viability and may be potentiated by the degree of chemical branching and intermolecular association (i.e. single/triple helix or randomly coil structures)\textsuperscript{11}.

Human controlled exposure studies\textsuperscript{12–15} provide a unique opportunity to simulate air pollution levels like those seen regularly in cities like Beijing, China\textsuperscript{16}, while allowing for experimental control of the level of exposure. We used a double-blind randomized cross-over trial of controlled human exposures to coarse and fine concentrated ambient particles (CAPs) to evaluate their effects on acute pulmonary and secondary systemic inflammatory responses. In addition, we assessed whether exposure to increased concentrations of CAPs-associated endotoxin and glucan explained the inflammatory response to CAPs exposure.

**METHODS**

**Study Participants**

We included 35 healthy non-smokers, aged 18–60 years, with no history of cardiovascular disease, hypertension (blood pressure > 140/90 mmHg) or diabetes. Subjects were not receiving treatment with cholesterol lowering medication or corticosteroids, and were free of respiratory tract infections for at least three weeks prior to exposure testing. Subjects were recruited from the University of Toronto Campus and surrounding area. The study was approved by the human research ethics committees of St. Michael’s Hospital, the University of Toronto, and Health Canada. All participants provided written informed consent before enrolling.

**Study Design & Exposure Assessment**

In a double-blind randomized cross-over design, participants were exposed to five exposures: 1) fine CAPs between 0.1 and 2.5 microns aerodynamic diameter (~250 μg/m\textsuperscript{3}); 2) coarse CAPs between 2.5 and 10 microns aerodynamic diameter (~200 μg/m\textsuperscript{3}); 3) a second coarse CAPs (~200 μg/m\textsuperscript{3}); 4) filtered air; and 5) medical air. Previous studies by our group had assessed the acute effects of fine CAPs\textsuperscript{17,18}. When we began this study, the effects of coarse CAPs were not well assessed. We added the second coarse exposure per subject to increase the power to assess effects on outcomes. The two coarse exposures were handled as separate treatments in the statistical analyses to increase variability and power. Each exposure lasted 130-minutes (120-minutes exposure plus an additional 10-minutes to complete all test measures), followed by a minimum two-week washout period before the next exposure. Controlled exposures were generated using high-flow (5,000 L/min) Harvard Ambient Particle Concentrators\textsuperscript{19–21}. These particle concentration systems were used to draw ambient particles from a 1.8 m high PM\textsubscript{10} inlet located, 10 m from a busy 4-lane downtown Toronto street with ~2,500 vehicles passing during the 130-minute exposure. Thus, traffic emissions were a major contributor to the ambient PM levels at this site. Ambient particle exposures were concentrated and adjusted through a dilution control system to deliver target concentrations of ~200 μg/m\textsuperscript{3} coarse CAPs and ~250 μg/m\textsuperscript{3} fine CAPs. The CAPs air stream was delivered directly to the subject seated inside a 4.9 m\textsuperscript{3} Lexan and steel tube frame enclosure, at rest and breathing freely (no mouthpiece) via an “oxygen type” face-mask covering his/her nose and mouth. The study design called for rest

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so there would be no interference with the cardiovascular measure of flow-mediated dilation (not included in this manuscript). The delivery system was designed so that there were no visual cues as to the exposure type while participants were seated in the chamber.

For filtered air exposures, the coarse concentrator was run with a high-efficiency particulate air (HEPA) filter placed in-line to remove the particles. For medical air exposures, compressed breathing-grade medical air was humidified to 30% relative humidity, passed through an in-line HEPA filter and delivered to the subject at a flow rate of 30 L/min. Medical air was selected because it is free of gaseous and particulate pollutants and odors.

The original study design (randomized block) only included the first four exposures (no medical air). However, interim analysis showed greater than expected physiological responses with filtered air, which was designed to serve as the control exposure and thus was expected to induce a negligible effect. We hypothesized that these responses may have been due to ambient gases such as volatile organic compounds (VOC) that can pass through the HEPA filter. Thus, a fifth exposure using medical air was added as a second additional control, although in the first 11 subjects it was always delivered to the subject as the last exposure in the series (i.e., not randomized), but then randomized in later subjects. This deviation from the randomized block design was considered in statistical analyses.

Endotoxin and glucan were both collected on polycarbonate membrane filters during coarse and fine CAPs and filtered air (but not medical air) exposures. Filters were placed in pyrogen-free 15 mL French square bottles containing 5 mL of Limulus Amebocyte Lysate (LAL) reagent water. After shaking for 20-minute and mixing for 1-minute with a vortex, samples were divided into two aliquots of 2.5 mL. To measure endotoxin, the sample was sonicated for 30-minutes at 26°C and then vortexed for another 1 min. The extract was analyzed for endotoxin using Pyrochrome and Glucashield reagents following manufacturer’s instructions (Associates of Cape Cod, Inc. [ACC], East Falmouth, Massachusetts). To measure glucan, 0.3 N NaOH was added to the sample, shaken on ice for 25-minutes, and diluted with 10 mM NaOH. The extract was analyzed for the amount of glucan using Glucatell reagent [ACC].

**Outcome Measures**

We collected induced sputum at the screening visit (median 21 days prior to the first exposure treatment) and at 24-hours post exposure. In this study, we used sputum total cell and neutrophil counts as outcomes. Venous blood was also collected from all study participants ~45-minutes prior to, and 3 and 24-hours after the start of each exposure. We evaluated total blood leukocyte and neutrophil counts as well as blood interleukin-6 (IL-6) and high-sensitivity C-reactive protein (hs-CRP) as markers of inflammation.

**Statistical Methods**

We first described the baseline characteristics of the study participants and the exposure concentrations (mass, endotoxin, and glucan) across the different treatments (coarse and fine CAPs and filtered/medical air). To account for the within-subject correlation in the outcome measures, while adjusting for daily physiological variability within subjects, we created new variables representing change. Therefore, blood outcomes were converted to: 1) 3-hour post
pre change; and 2) 24-hour post – pre change. Sputum measures were converted to the 24-hour post – screening visit change. However, since the screening visit was a median 21 days prior to the first exposure treatment, we also performed sensitivity analyses using 24-hour post sputum measures as a single measure. We assessed the outcome measure distributions for normality, and if skewed, we transformed the data, as appropriate, prior to further analyses.

We used linear mixed effects models to account for the within-subject correlation in responses between the exposure treatments. We first examined whether, relative to filtered air, coarse and fine CAPs and medical air exposures were independently associated with each outcome. We included the 4 exposure types (treatments) as a categorical variable, and used filtered air as the control, which was randomized for all subjects by design. We then examined whether, accounting for the exposure type, variations in bioaerosol (endotoxin, and glucan) concentrations were associated with the inflammatory outcomes. Due to the collinearity in bioaerosol concentrations, we assessed the effect of endotoxin and glucan in separate models. Coarse and fine CAPs-associated bioaerosols were initially grouped together. We subsequently used interaction terms between bioaerosol concentrations and CAPs size fraction to examine whether associations between bioaerosol exposures and outcomes were modified by CAPs size fraction.

All models were tested as follows: 1) unadjusted, assuming the randomized design efficiently accounted for measured and unmeasured confounders; 2) adjusting for exposure order (1st–5th; as in Table 3), to account for any potential stress or cumulative responses; and 3) adjusting for subjects’ age, gender, ethnicity, body mass index [BMI (kg/m\(^2\)], and season (categorical; 4-levels) of exposure treatment. Continuous covariates (age and BMI) were centered at their respective means. Lastly, sensitivity analyses were performed to ensure results were not due to any outliers, identified as the highest two/three exposure concentrations or outcomes (sputum/blood white cell and neutrophil counts).

RESULTS

Nineteen (54%) men and 16 (46%) women completed a total of 132 controlled exposure treatments. Seventeen (48%) were Asian, 16 (46%) were white, and 2 (6%) were black. Their mean (interquartile range) age and BMI were 27 (11) years and 23 (3) kg/m\(^2\), respectively.

Table 1 shows the distribution of the 132 controlled exposure treatments. While the coarse and fine CAPs concentrations were tightly controlled by design, there was residual variability in both exposures (interquartile range (IQR))\(_{coarse} = 35.8 \mu g/m^3; \ IQR_{fine} = 52.4 \mu g/m^3\). Glucan levels obtained were on average [median (IQR)] 4.6 (5.2) times higher than ambient for coarse CAPs exposures, and 4.4 (2.2) for fine CAPs exposures. Endotoxin levels were on average [median (IQR)] 5.8 (4.2) times higher than ambient for coarse CAPs exposures, and 7.7 (3.9) for fine CAPs exposures. No particulate mass, glucan or endotoxin were found in the filtered air. We did not measure bioaerosol concentrations in medical air.
At baseline, all subjects were apyrexial and showed no signs of infection (maximum total leukocyte counts = 8.9 × 10^9 cells/L; maximum blood neutrophil counts = 5.9 × 10^9 cells/L) or inflammation (maximum hs-CRP = 6.9 μg/mL). With the exception of up to a few marked responses in each outcome, blood and sputum outcome distributions (Table 2) were normally distributed. While the mean changes in blood and sputum outcomes appeared minimal, there was variability in the responses. However, we did not observe significant differences in subject characteristics (age, gender, ethnicity, or BMI) between those with increased or decreased responses (results not shown).

Table 3 presents the associations between the controlled exposure treatments and the change in total blood leukocyte responses. While there appeared to be a response to filtered air (the reference group) 3-hours post exposure in both the unadjusted model (intercept estimate = 0.50 × 10^9 cells/L [95% confidence interval (CI): 0.12, 0.87]) and in model 1 adjusting for exposure order (estimate = 0.62 × 10^9 cells/L [95% CI: 0.20, 1.05]), this was no longer statistically significant when we adjusted for potential confounders in model 2 (estimate = 0.60 × 10^9 cells/L [95% CI: -0.06, 1.26]). Relative to filtered air, coarse and fine CAPs and medical air were not significantly associated with 3-hour post-pre change blood leukocyte responses (model 2). However, an increase in total blood leukocytes 24-hours post exposure (Table 4, model 2) was significantly associated with coarse [estimate = 0.44 × 10^9 cells/L (95% CI: 0.01, 0.88)] and fine CAPs [estimate = 0.68 × 10^9 cells/L (95% CI: 0.19, 1.17)], but not medical air [estimate = 0.36 × 10^9 cells/L (95% CI: −0.20, 0.93)]. We performed sensitivity analyses by removing marked responses, and found consistent results in models with blood neutrophil responses (Supplemental Material, Table 1).

Adjusting for treatment type (Table 3, model 4), an interquartile increase in endotoxin (5.4 ng/m^3) was significantly associated [estimate = 0.38 × 10^9 cells/L (95% CI: 0.09, 0.68)] with an increase in blood leukocytes 3-hours post exposure. While an interquartile increase in endotoxin concentration was associated [estimate = 0.37 × 10^9 cells/L (95% CI: 0.12, 0.63)] with higher leukocytes 24-hours post exposure (Table 4, model 3), this association was no longer significant when we adjusted for treatment type (Table 4, model 4). In a model excluding medical air exposures to obtain the same number of observations as in model 4 (results not shown in tables), coarse [estimate = 0.52 × 10^9 cells/L (95% CI: 0.04, 1.00)] and fine CAPs [estimate = 0.74 × 10^9 cells/L (95% CI: 0.21, 1.27)] remained significantly associated with higher leukocyte levels 24-hours post exposure. However, when including endotoxin in model 4, the associations of coarse and fine CAPs became non-significant and the respective effect estimates were reduced by 40% (0.52 to 0.31 × 10^9 cells/L) and 35% (0.74 to 0.48 × 10^9 cells/L). The association between increases in endotoxin concentration and leukocyte responses 3-hours (p for interaction = 0.67) or 24-hours (p for interaction = 0.42) post exposure did not vary significantly by CAPs size fraction.

Variations in glucan concentrations were not associated with 3-hour or 24-hour post leukocyte responses. Relative to filtered air, fine CAPs exposures were associated [estimate = −0.62 μg/mL (95% CI: −1.04, −0.20), n = 132] with lower hs-CRP responses 24-hours post treatment (Supplemental Material, Table 2). This negative association did not remain significant [estimate = −0.23 μg/mL (95% CI: −0.62, 0.15), n = 128] after we removed four
marked responses presented (~2.6, 1.4, 2.2, and 3.7 μg/mL). We did not find any associations with blood IL-6 (Supplemental Material, Table 3) or sputum responses (Supplemental Material, Table 4).

DISCUSSION

In a double-blind randomized cross-over trial in 35 healthy adult subjects, coarse and fine ambient particle exposures were independently associated with an acute inflammatory response. The endotoxin content partially explained the inflammatory role of ambient particle exposures, and the effect did not differ between coarse and fine particles.

While we observed significant associations with systemic inflammatory responses (blood neutrophils), the lack of significant corollary findings in sputum may be due to insufficient power because of the small sample size. Nevertheless, human exposure studies of CAPs have generally not shown consistent results with induced sputum, with suggestions that systemic inflammation may be more pronounced than pulmonary responses. CAPs represent multiple sources of ambient pollutants rather than just DE emissions. While we controlled total mass levels in our study, we were limited by not accounting for particle composition which varies with time due to changes in source emissions and prevailing environmental conditions. CAPs composition also varies by study location, and therefore our results from Toronto may not represent what might be found elsewhere.

Ambient particulate matter constituents may include chemicals such as metals, organics, and biological materials from bacteria, viruses, and fungi. An in vitro study of rat alveolar macrophage (AM) cells found that endotoxin in urban air particles, but not in diesel particles, was responsible for inducing inflammatory cytokine expression. Humans may be more sensitive than animals to the effects of CAPs. Becker et al. (2005) exposed AM cells from healthy adult subjects aged 20 – 35 years to fine and coarse particles in vitro and found that the main proinflammatory response was driven by the coarse size fraction, where the majority (~90%) of the stimulatory material in inhalable PM is known to be found. This stimulatory material is mainly derived from biological sources, and includes microbes and allergens.

Alexis et al. (2006) exposed nine healthy subjects, on three separate occasions, to inhale either nebulized saline (0.9%, control), coarse PM collected from local ambient air in Chapel Hill, NC, that was heated (20 hours at 120°C) to inactivate biological material, or non-heated PM. Relative to saline, coarse PM exposure was associated with an increase in inflammatory polymorphonuclear leukocytes and macrophage mRNA TNF-α, an up-regulation of immune surface phenotypes on macrophages (mCD14, CD11b, HLA-DR), and increased phagocytosis, two to three hours post-inhalation. Biological inactivation was associated with lower mRNA TNF-α, phagocytosis, and cell surface marker responses. Analysis of ambient coarse PM from Chapel Hill, NC, showed that it contained 30% gram-negative bacteria, with the remainder mostly composed of gram-positive cocci and fungal spores (Penicillium, Cladosporium).
We measured slightly higher levels of endotoxin in fine than in coarse CAPs (Table 1). This may be due to the fact that subjects were, by design, exposed to higher concentrations of fine (~250 µg/m^3) than coarse (~200 µg/m^3) CAPs. Nevertheless, a separate epigenetic analysis of our study found coarse CAPs exposure to be associated with lowered toll-like receptor (TLR)-4 methylation, which can recognize the endotoxin component of coarse CAPs and trigger macrophages to release various inflammatory cytokines\(^{29}\).

Concentrated ambient endotoxin concentrations, irrespective of CAPs size fraction, were over seventeen-fold greater (geometric mean ~34 EU/m^3) than levels normally found in outdoor and indoor air (<2 EU/m^3)\(^{30, 31}\). Conversely, concentrated ambient glucan concentrations (geometric mean = 8 ng/m^3) were just above levels found in total unconcentrated PM in normal indoor and outdoor air\(^{24}\), perhaps explaining why we did not find associations with glucan exposures in our study. Levels greater than 5 ng/m^3 of glucan is generally associated with previous mold growth or water damage\(^{32}\); a villa with excessive mold growth had levels of up to 100 ng/m^3\(^{33}\). On the other hand, there may indeed be no health effects with glucan exposures.

A review of studies on the potential effects of glucan on airway inflammation showed mixed results\(^{34}\). This lack of consistency may be due to a number of reasons, such as small sample sizes, different exposure assessment methods, or lack of control for potential confounders or co-exposures such as endotoxin\(^{35-39}\). Furthermore, health effects vary by route of exposure (e.g., inhalation/oral) and type of glucan. The Glucatell reagent used to measure glucan concentrations are specific to (1→3)-β-D-glucan, and therefore, cross-reactivity with plant glucan that have 1→4 linkages (as found in barley) does not occur. However, factor G activation has a bias for higher molecular weight glucan and single-helix and randomly coiled conformers over triple-helix structures. The enzyme-linked immunosorbent assay (ELISA) method has been shown to be specific for fungi, (1→6) side-branched and (1→3)-β-D-glucan, as well as high molecular weight glucan, and may therefore be a better method for determining exposure to glucan likely to have important health effects\(^{40}\).

IL-6 is a cytokine that stimulates neutrophil production, the proliferation of B-lymphocytes, and the production of acute phase proteins (APP) by the liver. C-reactive protein (CRP) is an important APP that functions as a soluble pattern recognition receptor (PRR). PRRs, such as the family of TLRs, are found on antigen presenting cells and identify microbial conserved structures of pathogen-associated molecular patterns. The exposure duration (130-minutes) and length of follow-up (24-hours) was sufficient to elicit changes in IL-6\(^{20}\). The lack of significant associations with IL-6 or CRP in this study may therefore be due to a number of other reasons, including: 1) healthy subjects may not be representative of the population susceptible to the inflammatory effects of CAPs exposure; 2) other cytokines may have been released by macrophages, such as IL-1, IL-8, tumor necrosis factor alpha (TNF-α), and platelet-activating factor (PAF); and 3) there may indeed have been no effects to detect.

Our study was limited by the short exposure durations and follow-up periods, which may not be completely representative of the spatiotemporal variability in real-life exposures. Due to collinearity of endotoxin and glucan exposures, we were unable to assess any effect measure modification of the association between endotoxin and inflammation by glucan.
Furthermore, the selection of healthy adults may limit generalizability of study findings to susceptible sub-population. A limitation was that we did not evaluate differential deposition and we did not do nasal lavage (it would have been problematic to do both nasal lavage and sputum evaluation). It is possible that the weak associations of coarse particles with sputum leukocytes counts relate, in part, to deposition patterns. Our primary endpoints in the main study from which our analyses came from were cardiovascular. Finally, we were limited in that we did not have the exposures prior to the chamber exposure. Due to the randomization, the role of daily life exposures in the few days prior to each treatment were not expected to have had a differential impact on the association between exposures of varying size and health outcomes.

Despite these limitations, our study included a carefully standardized environment with well characterized exposures and physical activity levels. Our randomized study design enables subjects to serve as their own controls, thereby controlling for measured and unmeasured confounders. Circadian rhythms, physical activity, and stress must be taken into account when analyzing cytokines in peripheral blood; circadian rhythms were controlled for by standardizing the time of day when exposure treatments were performed; subjects were seated at rest during treatments; and stress was accounted for by adjusting for exposure order in statistical analyses. Study team members and subjects were both unaware of their exposure assignment in this double-blind study, thereby preventing the introduction of bias.

CONCLUSIONS

We have shown that short duration controlled human exposures to coarse and fine CAPs were independently associated with acute systemic inflammatory responses in healthy non-smoking adults. Endotoxin contributes to the inflammatory role of both coarse and fine particle air pollution.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>ACC</td>
<td>Associates of Cape Cod, Inc.</td>
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<tr>
<td>AM</td>
<td>alveolar macrophage</td>
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<tr>
<td>APP</td>
<td>acute phase proteins</td>
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BMI  body mass index
CAPs  concentrated ambient particles
CD14  cluster of differentiation 14
cells/L  cells per Liter
CI  confidence interval
CRP  C-reactive protein
DE  diesel exhaust
ELISA  enzyme-linked immunosorbent assay
EU/m³  endotoxin units per cubic meter
HEPA filter  high-efficiency particulate air filter
hs-CRP  high-sensitivity C-reactive protein
IL-1  interleukin-1
IL-6  interleukin-6
IL-8  interleukin-8
IQR  interquartile range
kg/m²  kilogram per square meter
L/min  Liters per minute
L/min/m²  Liters per minute per square meter
LAL  Limulus Amebocyte Lysate
LPS  lipopolysaccharide
m³  cubic meter
µg/mL  micrograms per milliliter
µg/m³  micrograms per cubic meter
mmHg  millimeters mercury
mM  millimolar
ng/m³  nanograms per cubic meter
pg/mL  picograms per milliliter
PM  particulate matter
PM₂.₅₋₀.₁  particulate matter with aerodynamic diameters between 0.1 and 2.5 microns
PM₂.₅₋₁₀  particulate matter with aerodynamic diameters between 2.5 and 10 microns
PM₁₀  particulate matter with aerodynamic diameters under 10 microns
n sample size
N normality (chemical concentration; millimoles per Liter)
NaOH sodium hydroxide
PAF platelet-activating factor
PRR pattern recognition receptor
TLR toll-like receptor
TNF-α tumor necrosis factor alpha
Vₑ minute ventilation
VOC volatile organic compounds

References


What this paper adds

• In healthy adults, controlled coarse as well as fine ambient particle exposures independently induced acute systemic inflammatory responses.
• Endotoxin contributes to the inflammatory role of particle air pollution.
• Knowledge of the particulate matter components responsible for the health effects observed in epidemiological studies is of importance for the development of targeted air pollution regulations.
Table 1

Exposure characteristics.

<table>
<thead>
<tr>
<th>Exposure Characteristic</th>
<th>TOTAL (all exposures)</th>
<th>Coarse CAPs (PM$_{2.5}$)</th>
<th>Fine CAPs (PM$_{2.5}$)</th>
<th>Filtered Air</th>
<th>Medical Air</th>
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<tr>
<td></td>
<td>N</td>
<td>median (μg/m³)</td>
<td>IQR</td>
<td>n</td>
<td>median (μg/m³)</td>
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<td>Particulate Mass Concentration (μg/m³)</td>
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<td>220.3</td>
<td>55</td>
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<td>Beta glucan (ng/m³)</td>
<td>80</td>
<td>9.0</td>
<td>17.7</td>
<td>40</td>
<td>13.0</td>
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<td>Endotoxin (ng/m³)</td>
<td>98</td>
<td>4.8</td>
<td>5.4</td>
<td>51</td>
<td>5.4</td>
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</table>

* Integrated gravimetric (filter sample) 130-minute exposure concentrations sampled from CAPs/filtered air airstream inlet to human chamber.

Abbreviations: CAPs = concentrated ambient particles; PM = particulate matter; n = number of observations; IQR = interquartile range
### Table 2

Outcome characteristics.

<table>
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<tr>
<th>OUTCOME</th>
<th>N</th>
<th>MEAN</th>
<th>MIN</th>
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<th>75th</th>
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<td>Total leukocytes (# cells × 10^9/L)</td>
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<td></td>
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<td>Pre treatment</td>
<td>132</td>
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<td>3.5</td>
<td>4.7</td>
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<td>6.2</td>
<td>8.9</td>
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<td>3-hour post treatment</td>
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<td>24-hour post treatment</td>
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<td>5.5</td>
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<td>6.1</td>
<td>9.8</td>
<td>1.4</td>
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<tr>
<td>3-hour - pre change</td>
<td>132</td>
<td>0.4</td>
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<td>−0.3</td>
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<tr>
<td>24-hour - pre change</td>
<td>131</td>
<td>0.1</td>
<td>−2.7</td>
<td>−0.6</td>
<td>0.1</td>
<td>0.5</td>
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</tr>
<tr>
<td>Neutrophils (# cells × 10^9/L)</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Pre treatment</td>
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<td>1.6</td>
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<td>3.6</td>
<td>5.9</td>
<td>1.2</td>
</tr>
<tr>
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<td>132</td>
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<td>1.6</td>
<td>2.6</td>
<td>3.2</td>
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<td>8.4</td>
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<td>3.0</td>
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<td>1.2</td>
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<tr>
<td>3-hour - pre change</td>
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<td>−0.1</td>
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<td>0.8</td>
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<tr>
<td>24-hour - pre change</td>
<td>131</td>
<td>0.0</td>
<td>−2.7</td>
<td>−0.5</td>
<td>0.0</td>
<td>0.4</td>
<td>4.0</td>
<td>0.9</td>
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<td>Interleukin-6 (pg/mL)</td>
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<tr>
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<td>0.0</td>
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<td>0.7</td>
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<td>0.4</td>
<td>0.6</td>
<td>0.9</td>
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<td>0.5</td>
</tr>
<tr>
<td>24-hour post treatment</td>
<td>121</td>
<td>1.1</td>
<td>0.0</td>
<td>0.5</td>
<td>0.8</td>
<td>1.1</td>
<td>6.0</td>
<td>0.6</td>
</tr>
<tr>
<td>3-hour - pre change</td>
<td>120</td>
<td>−0.1</td>
<td>−2.8</td>
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<td>−0.1</td>
<td>0.0</td>
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<tr>
<td>24-hour - pre change</td>
<td>121</td>
<td>0.1</td>
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<td>hs-CRP (μg/mL)</td>
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<tr>
<td>Pre treatment</td>
<td>121</td>
<td>1.3</td>
<td>0.0</td>
<td>0.1</td>
<td>0.5</td>
<td>2.0</td>
<td>6.9</td>
<td>1.9</td>
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<tr>
<td>3-hour post treatment</td>
<td>120</td>
<td>1.2</td>
<td>0.0</td>
<td>0.1</td>
<td>0.4</td>
<td>1.9</td>
<td>7.1</td>
<td>1.8</td>
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<tr>
<td>24-hour post treatment</td>
<td>121</td>
<td>1.3</td>
<td>0.0</td>
<td>0.1</td>
<td>0.4</td>
<td>1.9</td>
<td>10.0</td>
<td>1.8</td>
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<tr>
<td>3-hour - pre change</td>
<td>120</td>
<td>0.0</td>
<td>−1.9</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>2.1</td>
<td>0.1</td>
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<tr>
<td>24-hour - pre change</td>
<td>121</td>
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<td>−0.1</td>
<td>0.0</td>
<td>0.1</td>
<td>4.2</td>
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### INDUCED SPUTUM
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<thead>
<tr>
<th>OUTCOME</th>
<th>N</th>
<th>MEAN</th>
<th>MIN</th>
<th>25th</th>
<th>50th</th>
<th>75th</th>
<th>MAX</th>
<th>IQR</th>
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<tbody>
<tr>
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<td></td>
<td>Percentile</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td><strong>Total cells (# cells (\times 10^5)/mL)</strong></td>
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<td></td>
<td></td>
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<tr>
<td>Screening visit</td>
<td>10</td>
<td>9.7</td>
<td>2.6</td>
<td>7.1</td>
<td>9.2</td>
<td>13.2</td>
<td>16.6</td>
<td>6.1</td>
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<tr>
<td>24-hour post treatment</td>
<td>38</td>
<td>10.8</td>
<td>3.2</td>
<td>7.4</td>
<td>10.0</td>
<td>12.7</td>
<td>40.6</td>
<td>5.3</td>
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<tr>
<td>24-hour post - screening change</td>
<td>34</td>
<td>0.1</td>
<td>-10.2</td>
<td>-4.4</td>
<td>-0.7</td>
<td>5.0</td>
<td>19.1</td>
<td>9.4</td>
</tr>
<tr>
<td><strong>Neutrophils (# cells (\times 10^5)/mL)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Screening visit</td>
<td>10</td>
<td>3.1</td>
<td>1.4</td>
<td>1.4</td>
<td>2.8</td>
<td>4.5</td>
<td>6.9</td>
<td>3.1</td>
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<tr>
<td>24-hour post treatment</td>
<td>38</td>
<td>4.4</td>
<td>0.1</td>
<td>1.0</td>
<td>2.7</td>
<td>6.6</td>
<td>17.9</td>
<td>5.6</td>
</tr>
<tr>
<td>24-hour post - screening change</td>
<td>34</td>
<td>1.2</td>
<td>-4.1</td>
<td>-1.0</td>
<td>-0.1</td>
<td>2.5</td>
<td>13.6</td>
<td>3.5</td>
</tr>
</tbody>
</table>

Abbreviations: hs-CRP = high sensitivity C-reactive protein; IQR = interquartile range
Table 3

Associations between 130-min controlled human exposure treatments, bioaerosols, & 3-hour changes in blood total leukocytes.

<table>
<thead>
<tr>
<th>TREATMENT (categorical)</th>
<th>Unadjusted (n=132)</th>
<th>Model 1 (n=132)</th>
<th>Model 2 (n=132)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimate</td>
<td>95% CI</td>
<td>Estimate</td>
</tr>
<tr>
<td>Coarse CAPs</td>
<td>-0.10</td>
<td>(-0.46, 0.26)</td>
<td>-0.12</td>
</tr>
<tr>
<td>Fine CAPs</td>
<td>-0.04</td>
<td>(-0.44, 0.37)</td>
<td>-0.07</td>
</tr>
<tr>
<td>Medical Air</td>
<td>-0.39</td>
<td>(-0.83, 0.05)</td>
<td>-0.40</td>
</tr>
<tr>
<td>Filtered Air</td>
<td>ref</td>
<td>ref</td>
<td>ref</td>
</tr>
</tbody>
</table>

| Treatment group estimates are for the difference between filtered air and other treatment groups (coarse CAPs, fine CAPs or medical air) in leukocyte response (3-hours post minus pre exposure) to the treatment. |

Model 1 adjusts for exposure order (1st – 5th).

Models 2 – 6 adjusts for exposure order and subject characteristics:

- age (continuous), male gender, white ethnicity, BMI (continuous), and season (categorical; 4-levels).
- Note: age and BMI are centered at the mean.

Bioaerosol concentration (endotoxin or glucan) from coarse and fine CAPs. Estimates represent an interquartile increase in the exposure concentration (endotoxin = 5.4 ng/m$^3$; glucan = 17.7 ng/m$^3$)

Abbreviations: CI = confidence interval

‡ Bioaerosol concentration (endotoxin or glucan) from coarse and fine CAPs. Estimates represent an interquartile increase in the exposure concentration (endotoxin = 5.4 ng/m$^3$; glucan = 17.7 ng/m$^3$)

§ p ≤ 0.05

† p ≤ 0.01
### Table 4

Associations between 130-min controlled human exposure treatments, bioaerosols, & 24-hour changes in blood total leukocytes.

<table>
<thead>
<tr>
<th>TREATMENT (categorical)</th>
<th>24hr Post - Pre Change in Leukocytes (#cells * 10^9/L)</th>
<th>Unadjusted (n=132)</th>
<th>Model 1 (n=132)</th>
<th>Model 2 (n=132)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimate</td>
<td>95% CI</td>
<td>Estimate</td>
<td>95% CI</td>
</tr>
<tr>
<td>Coarse CAP</td>
<td>0.35</td>
<td>(−0.10, 0.80)</td>
<td>0.41</td>
<td>(−0.04, 0.87)</td>
</tr>
<tr>
<td>Fine CAP</td>
<td>0.67†</td>
<td>(0.16, 1.17)</td>
<td>0.71†</td>
<td>(0.19, 1.22)</td>
</tr>
<tr>
<td>Medical Air</td>
<td>0.21</td>
<td>(−0.33, 0.74)</td>
<td>0.35</td>
<td>(−0.24, 0.93)</td>
</tr>
<tr>
<td>Filtered Air</td>
<td>ref</td>
<td>ref</td>
<td>ref</td>
<td>ref</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TREATMENT (categorical)</th>
<th>Model 3 (n=98)</th>
<th>Model 4 (n=98)</th>
<th>Model 5 (n=80)</th>
<th>Model 6 (n=80)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimate</td>
<td>95% CI</td>
<td>Estimate</td>
<td>95% CI</td>
</tr>
<tr>
<td>Coarse CAP</td>
<td>-</td>
<td>-</td>
<td>0.31</td>
<td>(−0.23, 0.85)</td>
</tr>
<tr>
<td>Fine CAP</td>
<td>-</td>
<td>-</td>
<td>0.48</td>
<td>(−0.14, 1.10)</td>
</tr>
<tr>
<td>Medical Air</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Filtered Air</td>
<td>-</td>
<td>-</td>
<td>ref</td>
<td>ref</td>
</tr>
<tr>
<td>Endotoxin (ng/m^3)^‡</td>
<td>0.37‡</td>
<td>(0.12, 0.63)</td>
<td>0.25</td>
<td>(−0.05, 0.56)</td>
</tr>
<tr>
<td>Glucan (ng/m^3)^‡</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</tbody>
</table>

Treatment group estimates are for the difference between filtered air and other treatment groups (coarse CAPs, fine CAPs or medical air) in leukocyte response (24-hours post minus pre exposure) to the treatment.

Model 1 adjusts for exposure order (1st – 5th).
Models 2 – 6 adjusts for exposure order and subject characteristics:
- age (continuous), male gender, white ethnicity, BMI (continuous), and season (categorical; 4-levels).

Note: age and BMI are centered at the mean.

^‡ Bioaerosol concentration (endotoxin or glucan) from coarse and fine CAPs. Estimates represent an interquartile increase in the exposure concentration (endotoxin = 5.4 ng/m^3; glucan = 17.7 ng/m^3)

Abbreviations: CI = confidence interval

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† p ≤0.01