HHS Public Access

Author manuscript

Hypertension. Author manuscript; available in PMC 2016 September 01.

Published in final edited form as:

Hypertension. 2015 September ; 66(3): 509–516. doi:10.1161/HYPERTENSIONAHA.115.05342.

Endotoxin and β-1,3-D-Glucan in Concentrated Ambient Particles Induce Rapid Increase in Blood Pressure in Controlled Human Exposures

Jia Zhong[1,*], **Bruce Urch**[2], **Mary Speck**[2], **Brent A. Coull**[3], **Petros Koutrakis**[1], **Peter S. Thorne**[4], **James Scott**[2], **Ling Liu**[5], **Robert D. Brook**[6], **Behrooz Behbod**[1], **Heike Gibson**[1], **Frances Silverman**[2,7,8,9], **Murray A. Mittleman**[10], **Andrea A. Baccarelli**[1], and **Diane R. Gold**[1,11]

[1]Department of Environmental Health, Harvard T.H. Chan School of Public Health, Boston, Massachusetts, USA

[2]Division of Occupational & Environmental Health, Dalla Lana School of Public Health, University of Toronto, Toronto, Ontario, Canada

[3]Department of Biostatistics, Harvard T.H. Chan School of Public Health, Boston, Massachusetts, USA

[4]Department of Occupational and Environmental Health, University of Iowa, Iowa City, Iowa, USA

[5]Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, Ontario, Canada

[6]Division of Cardiovascular Medicine, University of Michigan, Ann Arbor, Michigan, USA

[7]Department of Medicine, University of Toronto, Toronto, Ontario, Canada

[8]Li Ka Shing Knowledge Institute, St Michael's Hospital, Toronto, Ontario, Canada

[9]Southern Ontario Centre for Atmospheric Aerosol Research, Toronto, Ontario, Canada

[10]Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, Massachusetts, USA

[11]Channing Laboratory, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, USA

Abstract

Short-term exposure to particulate matter (PM) is associated with increased blood pressure (BP) in epidemiological studies. Understanding the impact of specific PM components on BP is essential in developing effective risk-reduction strategies. We investigated the association between endotoxin and β-1,3-D-Glucan – two major biological PM components – and BP. We also examined whether vascular endothelial growth factor (VEGF), a vasodilatory inflammatory marker, modified these associations.

[*]Corresponding Author, Department of Environmental Health, Harvard T.H. Chan School of Public Health, Building-1 G11, 665 Huntington Avenue, Boston, MA 02115, Phone: (617)432-0478, Fax: (617)432-6913, jiazhong@mail.harvard.edu.

CONFLICT(S) OF INTEREST/DISCLOSURE(S)

None

We conducted a single-blind, randomized, crossover trial of controlled human exposure to Concentrated Ambient Particles (CAPs) with fifty healthy adults. Particle-associated-endotoxin and β-1,3-D-Glucan were sampled using polycarbonate-membrane-filters. Supine resting systolic (SBP) and diastolic BP (DBP) were measured pre-, 0.5-hr post-, and 20-hr post-exposure. Urine VEGF concentration was determined using enzyme-linked immunosorbant assay and creatinine-corrected. Exposures to endotoxin and β-1,3-D-Glucan for 130 minutes were associated with increases in BPs: At 0.5-hr post-exposure, every doubling in endotoxin concentration was associated with 1.73 mm Hg higher SBP (95% CI: 0.28, 3.18; *P*=0.02) and 2.07 mm Hg higher DBP (95% CI: 0.74, 3.39; *P*=0.003); every doubling in β-1,3-D-Glucan concentration was associated with 0.80 mm Hg higher SBP (95% CI: −0.07, 1.67; *P*=0.07) and 0.88 mm Hg higher DBP (95% CI: 0.09, 1.66; *P*=0.03). VEGF rose following CAP endotoxin exposure and attenuated the association between endotoxin and 0.5-hr post-exposure DBP ($P_{interaction}$=0.02).

In healthy adults, short-term endotoxin and β-1,3-D-Glucan exposures were associated with increased BP. Our findings suggest that the biological PM components contribute to PM-related cardiovascular outcomes, and post-exposure VEGF elevation might be an adaptive response that attenuates these effects.

Keywords

air pollution; blood pressure; vascular endothelial growth factor; endotoxin; β-1,3-D-Glucan

INTRODUCTION

Ambient particulate matter (PM) is a ubiquitous environmental health risk that contributes to about 3.2 million premature deaths per year worldwide.[1] The American Heart Association (AHA) has identified PM exposure as a primary contributor to cardiovascular morbidity and mortality, particularly due to rapid effects within hour or days after exposure peaks.[2] Increased blood pressure (BP) in response to air pollution peaks has been suggested as one of the primary intermediate outcomes contributing to acute air pollution-related cardiovascular disease (CVD) events.[2, 3] Identifying health effects of specific PM physico-chemical characteristics remains a critical gap in current knowledge. Ascertainment of how BP responses to particle mass differ by PM composition may aid in the development of targeted risk-reduction strategies.

Several mechanistic pathways have been proposed to account for the link between PM exposure and BP. For example, particles may interact with airway receptors and alter the autonomic nervous system balance. Recently, studies have emphasized the role of systemic inflammation in influencing PM-related changes in cardiovascular events, but few have focused on the biologic components of particles as the source of inflammatory pulmonary responses that may have downstream systemic and BP effects.[2]

PM consists of various major components, including inorganic and also biological materials (Supplemental Figure S1).[4, 5] Endotoxin – a lipopolysaccharide or oligosaccharide-protein complex originating from the outer membrane of Gram negative bacteria – is an important PM biological component because of its potent pro-inflammatory properties.[6, 7] Sources of

daily outdoor endotoxin are not well understood, but may include biologic components of roadway dust, agricultural dusts, airborne spores, and aqueous aerosols from industrial plants.[6] β-1,3-D-Glucan, another type of immune-modulating biological component in PM, is the most abundant form of polysaccharides found inside the fungal cell walls.[7] Epidemiological and animal studies have demonstrated that exposure to biological PM components induce airway and systemic inflammation, and are associated with immune-mediated respiratory tract and lung malignancy.[8, 9] In addition, studies have elucidated that high level of systemic endotoxin exposure associated with bacterial infections can trigger systemic vasodilation, hypotension, and diminish myocardial contractility.[10, 11] However, few studies have investigated the cardiovascular effect of pulmonary exposure to low level environmental particle-associated-endotoxin and β-1,3-D-Glucan.[12]

To investigate physiologic cardiovascular (including BP) responses to ambient particle-associated-endotoxin and β-1,3-D-Glucan, we conducted a single-blind, randomized, crossover trial of controlled human exposure to Concentrated Ambient Particles (CAPs). In a subset of study subjects, we previously demonstrated that exposures to fine and coarse CAPs were associated with increase in systolic BP (SBP).[13] In this study, we hypothesized that particle-associated-endotoxin and β-1,3-D-Glucan would be responsible for increased BP following CAPs exposure. We also examined whether vascular endothelial growth factor (VEGF), an important signal protein known to induce endothelium-dependent vasodilation,[14, 15] would modify susceptibility to BP effects following short-term ambient particle-associated-endotoxin and β-1,3-D-Glucan exposure.

METHODS

Study population

We recruited 50 healthy, 18–60 year old, non-smoking volunteers from the University of Toronto campus and surrounding area (See Supplemental Materials). 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

From November 2007 to March 2012, we conducted a single-blind, randomized, crossover controlled exposure study, as previously described.[15] The participants received up to five separate exposures in randomized orders: one exposure to fine CAPs (0.1-2.5 μm aerodynamic diameter, target concentration: 250 μg/m^3); two exposures to coarse CAPs (2.5-10 μm aerodynamic diameter, both with target concentration: 200 μg/m^3); one exposure to filtered air; and one exposure to medical air.[15, 16] Each exposure lasted 130 minutes and was followed by a minimum 2-week washout period before the next exposure.[15] The Harvard fine and coarse particle concentrators were used to generate CAPs, as previously described.[15, 17] The concentrator inlet was next to a heavy-traffic 4-lane street in downtown Toronto. The concentrated aerosol was mixed with particle free air using a dilution control system to deliver target-concentration CAPs. The composition of CAPs was not held fixed,

therefore the endotoxin and β-1,3-D-Glucan contents in CAPs varied across exposures. Finally, filtered air and medical air exposures were generated as previously described.[16]

Exposure assessment

During the exposure, particles were collected on polycarbonate membrane filters, and subsequently, were analyzed for endotoxin and β-1,3-D-Glucan using previously reported methods.[16] Endotoxin and β-1,3-D-Glucan samples were processed and analyzed in two labs using the same method, and the between-lab difference was taken into consideration in statistical analysis. Gravimetric determination of particle exposure mass concentration ($\mu g/m^3$) was acquired during each exposure. We also measured elemental composition of the fine and coarse CAPs (See Supplemental Materials).

BP and VEGF measurement

We measured supine resting SBP and diastolic BP (DBP) at three time points (pre, 0.5-hr post, and 20-hr post exposure) following a standardized protocol (See Supplemental Materials), as recommended by the AHA.[18] Pulse pressure (PP) was calculated as the difference between SBP and DBP. VEGF levels were analyzed on urine samples collected after overnight fasting (>8 hr), using previously described methods.[15]

Statistical methods

Covariates Selection and Model Assumption—For the analysis involving endotoxin and β-1,3-D-Glucan exposure, BP, and VEGF, we adjusted for covariates, selected based on prior knowledge and the existing literature, i.e., season (fall-winter/spring-summer), exposure types (coarse CAPs/fine CAPs/filtered air/medical air), filter configuration, CAP sampling location, and analysis lab. We also adjusted for the following potential additional influences on BP: age, body mass index (BMI), gender, chamber temperature and relative humidity.[15,16]

We performed a natural log transformation for endotoxin and β-1,3-D-Glucan by computing ln(concentration+1) to improve normality and stabilize the variance.[19] All endotoxin samples belonging to one participant were analyzed in the same lab, except for one participant. To account for the differences in data distribution at two analysis labs, the lab-specific standard deviation of ambient endotoxin measures was used to generate a correction factor, which was assumed to be similar as the timespan covered by each lab included all four seasons. Linear relationships were examined between BP and all independent variables and covariates, and no nonlinearity was observed. We scaled the effect estimates to the change in BP (mm Hg) per doubling the concentration of endotoxin/β-1,3-D-Glucan, which was well within the observed variation in exposure levels in the present study.

Linear mixed-effects models—To account for within-subject correlation in the outcome measures, a linear mixed-effects model (Model 1) was used to investigate the effect of the 130-min endotoxin exposure on BP. Random intercepts were assigned to each subject.

$$Y_{ij}=\beta_0+\beta_1 X_{1ij}+\beta_2 X_{2ij}+\ldots+\beta_p X_{pij}+b_i+\varepsilon_{ij} \quad \text{(Model 1)}$$

In the above model, Y_{ij} was the change in BP (ΔBP = post-exposure BP – pre-exposure BP) for participant i at exposure occasion j, β_0 was the overall intercept, and b_i was the separate random intercept for subject i with, $b_i \sim N(0, \Theta)$, $\varepsilon_{ij} \sim N(0, \sigma^2)$. X_{1ij} was the independent variable of interest. X_{2ij}–X_{pij} were the covariates, for participant i at measurement j. We further tested the effect modification by change in VEGF (ΔVEGF = post-exposure VEGF – pre-exposure VEGF) by fitting the main effect of ΔVEGF and an exposure*ΔVEGF interaction term in Model 1. A two tailed value of $P \leq 0.05$ was considered statistically significant. Analyses were performed using SAS 9.4 (SAS Institute, Cary NC).

RESULTS

Study population characteristics and exposure levels

Fifty participants completed a total of 176 controlled exposure experiments, out of which we obtained 139 and 115 measurements for endotoxin and β-1,3-D-Glucan, respectively. The median number of measurements per subject for endotoxin and β-1,3-D-Glucan was three (range: 1-5) and two (range: 1-5), respectively. All participants were healthy non-smokers aged between 18-60 years. Forty-four percent of the participants were white, 48% were Asian, and 8% were other races. Forty-six percent of the participants were male and 26% had BMI ≥25 (Table 1). During the study period, the endotoxin level varied from 0.03 to 21.30 ng/m^3 with a median of 2.50 ng/m^3; and the β-1,3-D-Glucan level ranged from 0.02 to 124.58 ng/m^3 with a median of 5.53 ng/m^3. There was no apparent difference in baseline BP status across subgroups, however, the baseline urine VEGF level varied across different age groups (Table 1). The potential effect of age was considered in the statistical analysis.

The ambient particle-associated-endotoxin level in CAPs differed by exposure types (Supplemental Table S1). Fine CAPs contained the highest endotoxin (median: 7.07 ng/m^3; interquartile range (IQR): 7.09 ng/m^3) and β-1,3-D-Glucan (median: 10.49 ng/m^3; IQR: 16.29 ng/m^3), while filtered air and medical air contained only trace amount of endotoxin and β-1,3-D-Glucan. Coarse CAPs on average contained less endotoxin (median: 4.30 ng/m^3; IQR: 5.15 ng/m^3) and β-1,3-D-Glucan levels (median: 6.56 ng/m^3; IQR: 17.82 ng/m^3) than fine CAPs, possibly due to a lower target concentration by design.

Endotoxin, β-1,3-D-Glucan, BP, and PP

Endotoxin exposure over the 130 minutes was associated with significantly higher SBP and DBP immediately post exposure, significantly higher DBP at 20-hr post exposure, non-significantly higher SBP at 20-hr post exposure, and non-significantly lower PP post-exposure (Table 2). Every doubling in endotoxin exposure was associated with 1.73 mm Hg (95% CI, 0.28 mm Hg, 3.18 mm Hg; P=0.02) increase in 0.5-hr post-exposure SBP, 0.84 mm Hg (95% CI, −0.75 mm Hg, 2.42 mm Hg; P=0.30) increase in 20-hr post-exposure SBP, 2.07 mm Hg (95% CI, 0.74 mm Hg; 3.39 mm Hg; P=0.003) increase in 0.5-hr post-exposure DBP, and 1.42 mm Hg (95% CI, 0.15 mm Hg, 2.70 mm Hg; P=0.03) increase in 20-hr post-exposure DBP. Exposure to β-1,3-D-Glucan over the 130 minuets was associated with higher 0.5-hr post-exposure SBP and DBP (Table 2). Every doubling in β-1,3-D-Glucan was associated with 0.80 mm Hg (95% CI, −0.07 mm Hg, 1.67 mm Hg; P=0.07) and 0.88 mm Hg (95% CI, 0.09 mm Hg, 1.66 mm Hg; P=0.03) increase in 0.5-hr post-exposure

SBP and DBP, respectively. No significant effect of β-1,3-D-Glucan exposure on PP and 20-hr post-exposure BPs was observed (Table 2).

Total exposure mass concentration, particle size, endotoxin, β-1,3-D-Glucan, and BP

CAPs exposures were controlled by design; however, there was small amount of variation in the actual CAP mass concentration (Supplemental Table S1). Therefore, we adjusted for the total exposure mass concentration to determine if the observed effects of endotoxin and β-1,3-D-Glucan on BP were partially due to CAP mass concentration. This adjustment resulted in only minor changes (Table 2). In addition, the association between endotoxin/β-1,3-D-Glucan and BP was not modified by exposure type, total exposure mass concentration, or ambient $PM_{2.5}$ level (data not shown).

Association of exposure to endotoxin/β-1,3-D-Glucan with VEGF

The 130-min endotoxin exposure was marginally associated with higher urine VEGF 0.5-hr post-exposure. For a doubling of the concentration of endotoxin, the estimated increase in urine VEGF was 12.78 pg/ml (95% CI, −0.79 pg/ml, 26.35 pg/ml; P=0.06). Per doubling the concentration of endotoxin was non-significantly associated with 3.61 pg/ml increase in urine VEGF at 20-hr post-exposure (95% CI, −12.57 pg/ml, 19.78 pg/ml; P=0.66). We did not observe a significant effect of β-1,3-D-Glucan on post-exposure urine VEGF.

Modification of endotoxin/β-1,3-D-Glucan association with BP by VEGF

The association between 130-min CAP endotoxin exposure and immediate post-exposure BP change was modified by the post-pre change of urine VEGF (ΔVEGF) ($P_{interaction}$=0.17 for SBP; $P_{interaction}$=0.02 for DBP) (Table 3). Endotoxin exposure had significant effects on BPs for individuals whose 0.5-hr post-exposure VEGF was lower than pre-exposure VEGF and those with a smaller amount of elevation in VEGF at 0.5-hr post-exposure (Q1, Q2, Q3); however, the effects were attenuated when there was a large elevation in 0.5-hr post-exposure VEGF (Q4). Analysis on β-1,3-D-Glucan did not show notable effect heterogeneity across ΔVEGF quartiles (Table 3).

Sensitivity Analyses

Exposures to CAP endotoxin and β-1,3-D-Glucan were measured by two labs; therefore, we performed analysis including only the data from one lab that handled about 70% of the samples to examine the robustness of our findings. This adjustment only resulted in minor changes in the conclusion, most likely due to compromised statistical power (Supplemental Table S2). In addition, we conducted analysis adjusting for race, fasting total cholesterol/high density lipoprotein cholesterol ratio, and weekday, and our results were stable and robust (Supplemental Table S3 and S4). We also examined the correlation matrix among endotoxin/β-1,3-D-Glucan and co-components to identify potential confounding variables by first assessing whether co-components were associated ($r \geq 0.6$) with either exposure, and none of the 19 co-components met this criterion (Supplemental Table S5).

DISCUSSION

This study on a single-blind, randomized, crossover controlled human exposure trial demonstrated the physiologic impact of short-term ambient particle-associated-endotoxin and β-1,3-D-Glucan on BP, and furthermore showed that endotoxin exposure was associated with increased urine VEGF level immediately following the exposure. In addition, novel findings suggested that individuals with negative or small increases in post-exposure urine VEGF level are more susceptible to elevated BP after endotoxin exposure, compared to those with more dramatic VEGF increases.

PM exposure contributes to cardiovascular morbidity and mortality, especially during acute exposure, as emphasized by the recent AHA statement on air pollution.[2] Short-term exposure to PM has been associated with rapidly increased BP in observational[19] and controlled human exposure studies to CAPs.[20-22] For example, effects of short-term ambient PM on BP have been observed in the general population,[23] healthy adults,[22] older adults,[24] cardiac disease patients,[19] and older adults with lung disease.[25] Our previous experiments of controlled human exposure to CAPs have reproducibly shown rapid increases in BP as early as two hours post-exposure.[13, 20-22, 26] However, identifying the PM component(s) responsible for PM-induced BP increase remained as a critical gap in current knowledge, and is considered an essential research priority to aid the effective risk-reduction strategies development. Studies have directed attention to the role of fine particles,[27] but studies on the role of biological PM components are lacking.

Although intravascular endotoxin is known to have a systemic vasodilatory effect,[14] in our study, inhalation of endotoxin was associated with a rise in BP. We previously demonstrated systemic pro-inflammatory endotoxin effects (i.e. increased blood leucocytes) that did not differ by particle size.[16] Systemic inflammation has been suggested as an essential mechanistic pathway linking acute adverse cardiovascular events following PM exposure.[2, 28] Thus, endotoxin and β-1,3-D-Glucan, two very potent inflammatory agents ubiquitously presenting in all PM size classes, are biologically plausible to be potential triggers of PM-induced cardiovascular pathology.[4] Recent studies showed that endotoxin stimulated airway inflammatory responses including granulocyte recruitment in healthy volunteers.[8, 29-31] Endotoxin activates the generation of inflammatory cytokines in human vascular endothelial cells, indicating that endotoxin-induced inflammation plays an important role in pathogenesis of vasculitis and arteriosclerosis.[32] Acute inhalation of high-dose endotoxin can cause immune failure symptoms such as systemic vasodilation – leading to hypotension and diminished myocardial contractility,[10, 11] while chronic inhalation of lower doses is associated with airway inflammation and respiratory organs impairment.[6, 8] On the other hand, β-1,3-D-Glucan – a component of cell walls in mold – can cause inflammation and oxidative stress in the respiratory tract, which may trigger systemic inflammation primarily through Dectin-1-mediated cellular responses.[33, 34]

In previous analysis in a subset of this study (with 15 subjects)[13] we have demonstrated that exposures to fine and coarse CAPs were significantly associated with higher SBP compared to medical air. However, when we extended the analyses to include all 50 participants, neither exposure type was significantly associated with increased BP. The difference in

conclusion was not due to demographic characteristics. However, it is possible that there are unmeasured characteristic differences in the two sets of population. Furthermore, the lack of a direct effect of exposure types in the full cohort may relate to the particle composition, which requires another investigative dimension to shed further light. In this present study – regardless of particle size (coarse *vs* fine) and particle mass – the endotoxin component of CAPs had the most reproducible effects on BP. Significant effects were also shown for β-1,3-D-Glucan, a measure of fungal exposure. Short-term exposure to endotoxin and β-1,3-D-Glucan not only immediately increased BP, but also produced a prolonged effect on heightened DBP lasting one day after exposure. This finding supports the hypothesis that chronic exposure to high-bioaerosol-content PM could lead to vascular responses that might accumulate over time and might not be completely reversible. Moreover, our results suggest that an important contributor of the vascular effect of PM might be its biological content.

This study further revealed that increased endotoxin exposure is linked with elevated urinary VEGF level, suggesting pro-inflammatory responses relevant to vascular function. We did not find significant interaction between β-1,3-D-Glucan and VEGF, indicating that the cardiovascular effects of β-1,3-D-Glucan and endotoxin are likely to act through different mechanisms. VEGF – a multifunctional angiogenic protein – regulates endothelial integrity, triggers endothelial cell proliferation and survival, and enhances inflammation.[35, 36] Brook and coauthors recently also observed increased number of circulating endothelial progenitor cells following 2-hr coarse PM exposure in a rural area.[37] Interestingly, the relation between VEGF and hypertension has been a topic of extensive debate because VEGF has not only proinflammatory and angiogenic effects, but also vasculoprotective/vasodilatory effects.[35, 38] Our data demonstrated that increased VEGF, an inflammatory response triggered following the endotoxin exposure, might reduce the effects of endotoxin exposure on increased BP in a progressive dose-dependent fashion. Taken together, our findings suggest that the increase in VEGF following short-term endotoxin exposure might be a compensatory humoral-vascular response to an acute “endothelial injury” that attenuates individual susceptibility to post-exposure BP increase.

This study has several strengths, including its single-blind, randomized, crossover controlled exposure design. Exposure misclassification, which is inherent in air pollution epidemiological studies, is minimized by the design that enables us to monitor the exposure at the individual level. We also conducted sensitivity analysis to rule out the potential impact of a laboratory effect. Although the outcome measurement error cannot be completely avoided, misclassification is likely non-differential (not associated with participants’ exposure status) and is expected to bias our result towards the null. The randomized crossover design also minimized the impact of time-invariant confounding. We also conducted analysis to evaluate the sensitivity of our results to covariate specification, and our results were stable and robust. The Harvard ambient particle concentrators do not concentrate the ambient gaseous pollutants such as ozone and sulfur dioxide, therefore, minimizing the confounding due to gaseous co-pollutants. In addition, all exposure experiments were conducted at the same time of the day to eliminate confounding due to diurnal variation. While residual confounding due to unmeasured variables is possible,

chances that the observed association and effect modification reflected bias resulting from confounding are minimized.

We acknowledge several other limitations in the present study. We only had 139 and 115 measures out of 176 total exposures for endotoxin and β-1,3-D-Glucan, respectively. Thirty-seven (21.0%) and 61 (34.7%) samples were excluded due to lack of filter samples for endotoxin/β-1,3-D-Glucan analysis. We compared the BP measures between those with endotoxin/β-1,3-D-Glucan data and those without, and no apparent difference was observed (data not shown). Therefore, selection bias due to informative missingness is unlikely. While we did not find that any of the elemental co-components confound the associations we found of endotoxin and β-1,3-D-Glucan with BP, it is possible that there are unmeasured CAP components or clusters of components that confounded the associations we report. In addition, our findings might not be generalizable to populations shown in epidemiologic studies to be at higher risk for pollution health effects (e.g., children, older adults, and individuals with preexisting cardiovascular disease).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

SOURCE(S) OF FUNDING

This publication was made possible by NIH P30ES005605, USEPA grant RD-83241601, RD-83479801, Health Canada's Clean Air Regulatory Agenda, Environment Canada, AllerGen NCE, NIH P01 ES009825, and NIH ES000002. Its contents are solely the responsibility of the grantee and do not necessarily represent the official views of the USEPA. Further, USEPA does not endorse the purchase of any commercial products or services mentioned in the publication.

REFERENCES

1. Lim SS, Vos T, Flaxman AD, et al. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990-2010: A systematic analysis for the global burden of disease study 2010. Lancet. 2012; 380:2224–2260. [PubMed: 23245609]
2. Brook RD, Rajagopalan S, Pope CA, Brook JR, Bhatnagar A, DiezRoux AV, Holguin F, Hong Y, Luepker RV, Mittleman MA. Particulate matter air pollution and cardiovascular disease: An update to the scientific statement from the American Heart Association. Circulation. 2010; 121:2331–2378. [PubMed: 20458016]
3. Brook RD, Bard RL, Morishita M, et al. Hemodynamic, autonomic, and vascular effects of exposure to coarse particulate matter air pollution from a rural location. Environmental Health Perspectives. 2014; 122:624–630. [PubMed: 24618231]
4. Harrison RM, Yin J. Particulate matter in the atmosphere: Which particle properties are important for its effects on health? The Science of the Total Environment. 2000; 249:85–101. [PubMed: 10813449]
5. Schwarze PE, Ovrevik J, Lag M, Refsnes M, Nafstad P, Hetland RB, Dybing E. Particulate matter properties and health effects: Consistency of epidemiological and toxicological studies. Human & Experimental Toxicology. 2006; 25:559–579. [PubMed: 17165623]
6. Reed CE, Milton DK. Endotoxin-stimulated innate immunity: A contributing factor for asthma. The Journal of Allergy and Clinical Immunology. 2001; 108:157–166. [PubMed: 11496229]

7. Rylander R. Endotoxin in the environment-exposure and effects. Journal of Endotoxin Research. 2002; 8:241–252. [PubMed: 12230914]
8. Jagielo PJ, Thorne PS, Watt JL, Frees KL, Quinn TJ, Schwartz DA. Grain dust and endotoxin inhalation challenges produce similar inflammatory responses in normal subjects. Chest. 1996; 110:263–270. [PubMed: 8681637]
9. Soukup JM, Becker S. Human alveolar macrophage responses to air pollution particulates are associated with insoluble components of coarse material, including particulate endotoxin. Toxicology and Applied Pharmacology. 2001; 171:20–26. [PubMed: 11181108]
10. Annane D, Bellissant E, Cavaillon JM. Septic shock. Lancet. 2005; 365:63–78. [PubMed: 15639681]
11. Landry DW, Levin HR, Gallant EM, Ashton RC Jr. Seo S, D'Alessandro D, Oz MC, Oliver JA. Vasopressin deficiency contributes to the vasodilation of septic shock. Circulation. 1997; 95:1122–1125. [PubMed: 9054839]
12. Douwes J, Thorne P, Pearce N, Heederik D. Bioaerosol health effects and exposure assessment: Progress and prospects. The Annals of Occupational Hygiene. 2003; 47:187–200. [PubMed: 12639832]
13. Bellavia A, Urch B, Speck M, Brook RD, Scott JA, Albetti B, Behbod B, North M, Valeri L, Bertazzi PA, Silverman F, Gold D, Baccarelli AA. DNA hypomethylation, ambient particulate matter, and increased blood pressure: Findings from controlled human exposure experiments. Journal of the American Heart Association. 2013; 2:e000212. [PubMed: 23782920]
14. Ku DD, Zaleski JK, Liu S, Brock TA. Vascular endothelial growth factor induces EDRF-dependent relaxation in coronary arteries. American Journal of Physiology. 1993; 265:H586–592. [PubMed: 8368362]
15. Liu L, Urch B, Poon R, Szyszkowicz M, Speck M, Gold DR, Wheeler AJ, Scott JA, Brook JR, Thorne PS, Silverman FS. Effects of ambient coarse, fine, and ultrafine particles and their biological constituents on systemic biomarkers: A controlled human exposure study [published online Janurary 16 2015]. Environmental Health Perspectives. 2015 doi:10.1289/ehp.1408387. Available at: http://ehp.niehs.nih.gov/1408387.
16. Behbod B, Urch B, Speck M, Scott JA, Liu L, Poon R, Coull B, Schwartz J, Koutrakis P, Silverman F, Gold DR. Endotoxin in concentrated coarse and fine ambient particles induces acute systemic inflammation in controlled human exposures. Occupational and Environmental Medicine. 2013; 70:761–767. [PubMed: 24143017]
17. Demokritou P, Gupta T, Ferguson S, Koutrakis P. Development of a high-volume concentrated ambient particles system (CAPs) for human and animal inhalation toxicological studies. Inhalation Toxicology. 2003; 15:111–129. [PubMed: 12528042]
18. Pickering TG, Hall JE, Appel LJ, Falkner BE, Graves J, Hill MN, Jones DW, Kurtz T, Sheps SG, Roccella EJ. Recommendations for blood pressure measurement in humans and experimental animals: Part 1: Blood pressure measurement in humans: A statement for professionals from the subcommittee of professional and public education of the american heart association council on high blood pressure research. Hypertension. 2005; 45:142–161. [PubMed: 15611362]
19. Zanobetti A, Canner MJ, Stone PH, Schwartz J, Sher D, Eagan-Bengston E, Gates KA, Hartley LH, Suh H, Gold DR. Ambient pollution and blood pressure in cardiac rehabilitation patients. Circulation. 2004; 110:2184–2189. [PubMed: 15466639]
20. Urch B, Silverman F, Corey P, Brook JR, Lukic KZ, Rajagopalan S, Brook RD. Acute blood pressure responses in healthy adults during controlled air pollution exposures. Environmental Health Perspectives. 2005; 113:1052–1055. [PubMed: 16079078]
21. Brook RD, Urch B, Dvonch JT, et al. Insights into the mechanisms and mediators of the effects of air pollution exposure on blood pressure and vascular function in healthy humans. Hypertension. 2009; 54:659–667. [PubMed: 19620518]
22. Brook RD, Brook JR, Urch B, Vincent R, Rajagopalan S, Silverman F. Inhalation of fine particulate air pollution and ozone causes acute arterial vasoconstriction in healthy adults. Circulation. 2002; 105:1534–1536. [PubMed: 11927516]

23. Ibald-Mulli A, Stieber J, Wichmann HE, Koenig W, Peters A. Effects of air pollution on blood pressure: A population-based approach. American Journal of Public Health. 2001; 91:571–577. [PubMed: 11291368]
24. Harrabi I, Rondeau V, Dartigues JF, Tessier JF, Filleul L. Effects of particulate air pollution on systolic blood pressure: A population-based approach. Environmental Research. 2006; 101:89–93. [PubMed: 16563371]
25. Chuang KJ, Chan CC, Shiao GM, Su TC. Associations between submicrometer particles exposures and blood pressure and heart rate in patients with lung function impairments. Journal of Occupational and Environmental Medicine. 2005; 47:1093–1098. [PubMed: 16282869]
26. Urch B, Brook JR, Wasserstein D, Brook RD, Rajagopalan S, Corey P, Silverman F. Relative contributions of $PM_{2.5}$ chemical constituents to acute arterial vasoconstriction in humans. Inhalation Toxicology. 2004; 16:345–352. [PubMed: 15204750]
27. Brook RD. You are what you breathe: Evidence linking air pollution and blood pressure. Current Hypertension Reports. 2005; 7:427–434. [PubMed: 16386198]
28. Stone PH, Godleski JJ. First steps toward understanding the pathophysiologic link between air pollution and cardiac mortality. American Heart Journal. 1999; 138:804–807. [PubMed: 10539808]
29. Hernandez ML, Herbst M, Lay JC, Alexis NE, Brickey WJ, Ting JP, Zhou H, Peden DB. Atopic asthmatic patients have reduced airway inflammatory cell recruitment after inhaled endotoxin challenge compared with healthy volunteers. Journal of Allergy and Clinical Immunology. 2012; 130:869–876. [PubMed: 22770265]
30. Hernandez ML, Harris B, Lay JC, Bromberg PA, Diaz-Sanchez D, Devlin RB, Kleeberger SR, Alexis NE, Peden DB. Comparative airway inflammatory response of normal volunteers to ozone and lipopolysaccharide challenge. Inhalation Toxicology. 2010; 22:648–656. [PubMed: 20540623]
31. Dillon MA, Harris B, Hernandez ML, Zou B, Reed W, Bromberg PA, Devlin RB, Diaz-Sanchez D, Kleeberger S, Zhou H, Lay JC, Alexis NE, Peden DB. Enhancement of systemic and sputum granulocyte response to inhaled endotoxin in people with the GSTM1 null genotype. Occupational and Environmental Medicine. 2011; 68:783–785. [PubMed: 21441173]
32. Libby P, Ordovas JM, Auger KR, Robbins AH, Birinyi LK, Dinarello CA. Endotoxin and tumor necrosis factor induce Interleukin-1 gene expression in adult human vascular endothelial cells. The American Journal of Pathology. 1986; 124:179–185. [PubMed: 3526909]
33. Thorn J, Rylander R. Airways inflammation and glucan in a rowhouse area. American Journal of Respiratory and Critical Care Medicine. 1998; 157:1798–1803. [PubMed: 9620908]
34. Brown GD, Herre J, Williams DL, Willment JA, Marshall AS, Gordon S. Dectin-1 mediates the biological effects of Beta-glucans. The Journal of Experimental Medicine. 2003; 197:1119–1124. [PubMed: 12719478]
35. Zhao Q, Ishibashi M, Hiasa K, Tan C, Takeshita A, Egashira K. Essential role of vascular endothelial growth factor in angiotensin II-induced vascular inflammation and remodeling. Hypertension. 2004; 44:264–270. [PubMed: 15262905]
36. Marumo T, Schini-Kerth VB, Busse R. Vascular endothelial growth factor activates nuclear factor-kappab and induces monocyte chemoattractant protein-1 in bovine retinal endothelial cells. Diabetes. 1999; 48:1131–1137. [PubMed: 10331420]
37. Brook RD, Bard RL, Kaplan MJ, Yalavarthi S, Morishita M, Dvonch JT, Wang L, Yang HY, Spino C, Mukherjee B, Oral EA, Sun Q, Brook JR, Harkema J, Rajagopalan S. The effect of acute exposure to coarse particulate matter air pollution in a rural location on circulating endothelial progenitor cells: Results from a randomized controlled study. Inhalation Toxicology. 2013; 25:587–592. [PubMed: 23919441]
38. Morishita R. Is vascular endothelial growth factor a missing link between hypertension and inflammation? Hypertension. 2004; 44:253–254. [PubMed: 15262906]

PERSPECTIVES

Our results provide for the first time experimental evidence showing that in healthy adults short-term exposure to the endotoxin and β-1,3-D-Glucan components of CAPs were associated with increases in SBP and DBP, which, for endotoxin, was partly ameliorated by a rise in VEGF. The functional and taxonomic definition of the CAP-associated microbes may provide further insight into the physiologic effects of CAPs and may help guide in targeted regulation of particles and their sources for health improvement.

NOVELTY AND SIGNIFICANCE

1) What is new?

For the first time, we investigated the association between endotoxin and β-1,3-D-Glucan – two major biological PM components – and BP in controlled human exposure experiments.

2) What is relevant?

Our results suggest that 1). An important determinant of the vascular effect of PM is its biological content, which would aid the development of effective targeted risk-reduction strategy; 2). VEGF elevation might be a compensatory humoral-vascular response to an acute “endothelial injury” that attenuates individual susceptibility to post-exposure BP increase.

Summary

Short-term exposures to endotoxin and β-1,3-D-Glucan were associated with increased BP in a randomized crossover trial of controlled human exposure to Concentrated Ambient Particles. Post-exposure VEGF elevation following endotoxin exposure attenuates the effect of endotoxin on BP.

Table 1

Baseline characteristics of study participants (n=50)

Characteristics	n (%)	SBP Mean (SD)	DBP Mean (SD)	PP Mean (SD)	VEGF Mean (SD)
Age (years)					
18-29	33 (66)	106.0 (9.4)	60.5 (5.6)	45.5 (7.0)	59.3 (57.7)
30-39	9 (18)	106.8 (11.3)	63.6 (8.9)	43.2 (5.4)	108.5 (88.6)
40-60	8 (16)	103.8 (8.9)	62.8 (7.8)	41.1 (2.4)	15.4 (22.2)
Gender					
Male	23 (46)	111.4 (8.2)	62.9 (6.9)	48.5 (5.0)	43.2 (45.7)
Female	27 (57)	101.0 (8.0)	60.1 (6.2)	40.9 (5.2)	75.2 (75.1)
Race					
White	22 (44)	105.9 (8.7)	61.1 (6.9)	44.8 (6.0)	63.6 (69.3)
Asian	24 (48)	105.5 (11.1)	61.4 (7.0)	44.1 (7.1)	57.0 (62.9)
Other	4 (8)	107.0 (3.1)	62.8 (1.7)	44.3 (3.3)	61.6 (63.2)
Body Mass Index (kg/m^2)					
<25	37 (74)	105.0 (9.5)	60.9 (6.9)	44.1 (6.2)	62.3 (68.6)
≥25	13 (26)	107.9 (9.7)	62.7 (5.6)	45.2 (6.9)	54.4 (53.7)

SBP and DBP indicate systolic and diastolic blood pressure, respectively; PP indicates pulse pressure; n indicates the number of subjects; SD indicates standard deviation; VEGF indicates vascular endothelial growth factor.

Table 2

Change in blood pressure (BP) per doubling the concentration in short-term (130 min) endotoxin and β-1,3-D-Glucan exposure

		Original model*			Adjusted for total exposure mass concentration†		
Outcome	**Time**	**Estimate**	**95% CI**	***P***	**Estimate**	**95% CI**	***P***
			Endotoxin				
ΔSBP	0.5h Post Exposure	1.73	0.28 to 3.18	0.02	2.01	0.49 to 3.54	0.01
ΔSBP	20h Post Exposure	0.84	−0.75 to 2.42	0.30	1.20	−0.46 to 2.86	0.16
ΔDBP	0.5h Post Exposure	2.07	0.74 to 3.39	0.003	2.00	0.65 to 3.35	0.004
ΔDBP	20h Post Exposure	1.42	0.15 to 2.70	0.03	1.46	0.11 to 2.81	0.03
ΔPP	0.5h Post Exposure	−0.27	−1.63 to 1.10	0.70	−0.02	−1.43 to 1.40	0.98
ΔPP	20h Post Exposure	−0.57	−1.90 to 0.76	0.40	−0.24	−1.63 to 1.15	0.73
			β-1,3-D-Glucan				
ΔSBP	0.5h Post Exposure	0.80	−0.07 to 1.67	0.07	0.84	−0.05 to 1.74	0.06
ΔSBP	20h Post Exposure	0.22	−0.75 to 1.20	0.65	0.37	−0.63 to 1.37	0.46
ΔDBP	0.5h Post Exposure	0.88	0.09 to 1.66	0.03	0.87	0.07 to 1.67	0.03
ΔDBP	20h Post Exposure	0.23	−0.55 to 1.00	0.56	0.23	−0.57 to 1.03	0.57
ΔPP	0.5h Post Exposure	0.09	−0.72 to 0.91	0.82	0.16	−0.67 to 1.00	0.70
ΔPP	20h Post Exposure	0.01	−0.85 to 0.88	0.98	0.16	−0.72 to 1.04	0.72

SBP and DBP indicate systolic and diastolic BP, respectively; PP indicates pulse pressure; CI indicates confidence interval.

*Results were adjusted for season, exposure type, age, body mass index, gender, chamber temperature and relative humidity, filter configuration, sampling location, and analysis lab.

†Results were adjusted for all the covariates listed above, and total exposure mass concentration.

Hypertension. Author manuscript; available in PMC 2016 September 01.

Table 3

Effect modification by vascular endothelial growth factor (VEGF) on the association between short-term (130 min) endotoxin or β-1,3-D-Glucan exposure and blood pressure (BP)

	0.5h Post Exposure			20h Post Exposure		
ΔVEGF	**Estimate***	**95% CI**	***P***	**Estimate ***	**95% CI**	***P***
Endotoxin exposure and ΔSBP						
Midpoint of Q1	2.71	0.77 to 4.65	0.007	1.49	−0.58 to 3.55	0.16
Midpoint of Q2	2.18	0.62 to 3.74	0.007	1.21	−0.46 to 2.87	0.15
Midpoint of Q3	1.94	0.46 to 3.42	0.01	1.08	−0.52 to 2.67	0.18
Midpoint of Q4	1.24	−0.47 to 2.95	0.15	0.70	−1.18 to 2.58	0.46
$P_{interaction}$			0.17			0.50
Endotoxin exposure and ΔDBP						
Midpoint of Q1	3.46	1.75 to 5.18	0.0001	1.96	0.28 to 3.63	0.02
Midpoint of Q2	2.64	1.26 to 4.03	0.0003	1.66	0.31 to 3.01	0.02
Midpoint of Q3	2.26	0.93 to 3.59	0.001	1.53	0.23 to 2.82	0.02
Midpoint of Q4	1.18	−0.38 to 2.73	0.14	1.14	−0.39 to 2.66	0.14
$P_{interaction}$			0.02			0.39
β-1,3-D-Glucan exposure and ΔSBP						
Midpoint of Q1	1.08	−0.02 to 2.19	0.05	0.12	−1.12 to 1.36	0.84
Midpoint of Q2	0.90	−0.01 to 1.80	0.05	0.21	−0.81 to 1.22	0.68
Midpoint of Q3	0.81	−0.07 to 1.68	0.07	0.25	−0.73 to 1.23	0.61
Midpoint of Q4	0.56	−0.47 to 1.59	0.28	0.36	−0.81 to 1.53	0.54
$P_{interaction}$			0.40			0.73
β-1,3-D-Glucan exposure and ΔDBP						
Midpoint of Q1	1.07	0.06 to 2.08	0.04	0.25	−0.75 to 1.24	0.62
Midpoint of Q2	0.94	0.12 to 1.76	0.03	0.24	−0.58 to 1.05	0.56
Midpoint of Q3	0.88	0.09 to 1.68	0.03	0.23	−0.56 to 1.02	0.56
Midpoint of Q4	0.71	−0.23 to 1.65	0.14	0.22	−0.72 to 1.15	0.65
$P_{interaction}$			0.52			0.96

SBP and DBP indicate systolic and diastolic BP, respectively; CI indicates confidence interval; ΔVEGF indicates the post-pre VEGF elevation; Q1, Q2, Q3, and Q4 indicate the 1^{st}, 2^{nd}, 3^{rd}, and 4^{th} quartile.

Midpoint of Q1: −49.58 pg/ml; Midpoint of Q2: −6.99 pg/ml; Midpoint of Q3: 12.67 pg/ml; Midpoint of Q4: 69.14 pg/ml.

*The effect estimates were obtained from the mixed effect model, representing the change in BP (mm Hg) per doubling the concentration of endotoxin or β-1,3-D-Glucan in the corresponding subgroup. Please refer to Table 2 for the overall effect estimates.

Results were adjusted for season, exposure type, age, body mass index, gender, chamber temperature and relative humidity, filter configuration, sampling location, and analysis lab.