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

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See Editorial Commentary, pp 469–471

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, a vasodilatory inflammatory marker, modified these associations. We conducted a single-blind, randomized, crossover trial of controlled human exposure to concentrated ambient particles with 50 healthy adults. Particle-associated-endotoxin and β-1,3-D-Glucan were sampled using polycarbonate-membrane-filters. Supine resting systolic BP and diastolic BP were measured pre-, 0.5-hour post-, and 20-hour postexposure. Urine vascular endothelial growth factor 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-hour postexposure, every doubling in endotoxin concentration was associated with 1.73 mm Hg higher systolic BP (95% confidence interval, 0.28, 3.18; P=0.02) and 2.07 mm Hg higher diastolic BP (95% confidence interval, 0.74, 3.39; P=0.003); every doubling in β-1,3-D-Glucan concentration was associated with 0.80 mm Hg higher systolic BP (95% confidence interval, −0.07, 1.67; P=0.07) and 0.88 mm Hg higher diastolic BP (95% confidence interval, 0.09, 1.66; P=0.03). Vascular endothelial growth factor rose after concentrated ambient particle endotoxin exposure and attenuated the association between endotoxin and 0.5-hour postexposure diastolic BP ($P_{interaction}$=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 postexposure vascular endothelial growth factor elevation might be an adaptive response that attenuates these effects. (***Hypertension*. 2015;66:509-516. DOI: 10.1161/HYPERTENSIONAHA.115.05342.)● Online Data Supplement**

Key Words: air pollution ■ blood pressure ■ endotoxin ■ β-1,3-d-Glucan ■ vascular endothelial growth factor

Ambient particulate matter (PM) is a ubiquitous environmental health risk that contributes to ≈3.2 million premature deaths per year worldwide.[1] The American Heart Association has identified PM exposure as a primary contributor to cardiovascular morbidity and mortality, particularly because of 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 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

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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 biological 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 (Figure S1 in the online-only Data Supplement).[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 proinflammatory properties.[6,7] Sources of daily outdoor endotoxin are not well understood, but may include biological 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 physiological 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 after 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 after short-term ambient particle-associated-endotoxin and β-1,3-D-Glucan exposure.

Methods

Study Population

We recruited 50 healthy, 18- to 60-year-old, nonsmoking volunteers from the University of Toronto campus and surrounding area (see online-only Data Supplement). 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 ≤5 separate exposures in randomized orders: 1 exposure to fine CAPs (0.1–2.5 μm aerodynamic diameter, target concentration: 250 μg/m^3); 2 exposures to coarse CAPs (2.5–10 μm aerodynamic diameter, both with target concentration: 200 μg/m^3); 1 exposure to filtered air; and 1 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 2 laboratories using the same method, and the between-laboratory difference was taken into consideration in statistical analysis. Gravimetric determination of particle exposure mass concentration (μg/m^3) was acquired during each exposure. We also measured elemental composition of the fine and coarse CAPs (see online-only Data Supplement).

BP and VEGF Measurement

We measured supine resting SBP and diastolic BP (DBP) at 3 time points (pre, 0.5-hour post-, and 20-hour postexposure) following a standardized protocol (see online-only Data Supplement), as recommended by the American Heart Association.[18] Pulse pressure was calculated as the difference between SBP and DBP. VEGF levels were analyzed on urine samples collected after overnight fasting (>8 hours), 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, that is, season (fall-winter/spring-summer), exposure types (coarse CAPs/fine CAPs/filtered air/medical air), filter configuration, CAP sampling location, and analysis laboratory. We also adjusted for the following potential additional influences on BP: age, body mass index, sex, 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 laboratory, except for one participant. To account for the differences in data distribution at 2 analysis laboratories, the laboratory-specific standard deviation of ambient endotoxin measures was used to generate a correction factor, which was assumed to be similar because the timespan covered by each laboratory included all 4 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-minute endotoxin exposure on BP. Random intercepts were assigned to each subject.

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

In the above model, Y_{ij} was the change in BP (ΔBP=postexposure 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, $b_i \approx N(0, \theta)$, $\varepsilon_{ij} \approx N(0, \sigma^2)$. 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=postexposure VEGF–preexposure VEGF) by fitting the main effect of ΔVEGF and an exposure×ΔVEGF interaction term in Model 1. A 2-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 3 (range, 1–5) and 2 (range, 1–5), respectively. All participants were healthy nonsmokers aged between 18 and 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 body mass index ≥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 (Table S1). Fine CAPs contained the highest endotoxin (median, 7.07 ng/m^3; interquartile range, 7.09 ng/m^3) and β-1,3-D-Glucan (median, 10.49 ng/m^3; interquartile range, 16.29 ng/m^3), whereas 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; interquartile range, 5.15 ng/m^3) and β-1,3-D-Glucan levels (median, 6.56 ng/m^3; interquartile range, 17.82 ng/m^3) than fine CAPs, possibly because of a lower target concentration by design.

Endotoxin, β-1,3-D-Glucan, BP, and Pulse Pressure

Endotoxin exposure over the 130 minutes was associated with significantly higher SBP and DBP immediately post exposure, significantly higher DBP at 20-hour postexposure, nonsignificantly higher SBP at 20-hour postexposure, and nonsignificantly lower pulse pressure postexposure (Table 2). Every doubling in endotoxin exposure was associated with 1.73 mm Hg (95% confidence interval [CI], 0.28 mm Hg, 3.18 mm Hg; P=0.02) increase in 0.5-hour postexposure SBP, 0.84 mm Hg (95% CI, −0.75 mm Hg, 2.42 mm Hg; P=0.30) increase in 20-hour postexposure SBP, 2.07 mm Hg (95% CI, 0.74 mm Hg, 3.39 mm Hg; P=0.003) increase in 0.5-hour postexposure DBP, and 1.42 mm Hg (95% CI, 0.15 mm Hg, 2.70 mm Hg; P=0.03) increase in 20-hour postexposure DBP. Exposure to β-1,3-D-Glucan over the 130 minutes was associated with higher 0.5-hour postexposure 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-hour postexposure SBP and DBP, respectively. No significant effect of β-1,3-D-Glucan exposure on pulse pressure and 20-hour postexposure 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

Table 1. Baseline Characteristics of Study Participants (n=50)

Characteristics	*n*, %	SBP, Mean (SD)	DBP, Mean (SD)	PP, Mean (SD)	VEGF, Mean (SD)
Age, y					
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)
Sex					
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)

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

Table 2. Change in Blood Pressure (BP) per Doubling the Concentration in Short-Term (130 minutes) Endotoxin and β-1,3-D-Glucan Exposure

		Original Model*			Adjusted for Total Exposure Mass Concentration†		
Outcome	Time	Estimate	95% CI	*P* Value	Estimate	95% CI	*P* Value
Endotoxin							
ΔSBP	0.5 h post exposure	1.73	0.28–3.18	0.02	2.01	0.49–3.54	0.01
ΔSBP	20 h post exposure	0.84	−0.75 to 2.42	0.30	1.20	−0.46 to 2.86	0.16
ΔDBP	0.5 h post exposure	2.07	0.74–3.39	0.003	2.00	0.65–3.35	0.004
ΔDBP	20 h post exposure	1.42	0.15–2.70	0.03	1.46	0.11–2.81	0.03
ΔPP	0.5 h post exposure	−0.27	−1.63 to 1.10	0.70	-0.02	−1.43 to 1.40	0.98
ΔPP	20 h 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	0.5 h post exposure	0.80	−0.07 to 1.67	0.07	0.84	−0.05 to 1.74	0.06
ΔSBP	20 h post exposure	0.22	−0.75 to 1.20	0.65	0.37	−0.63 to 1.37	0.46
ΔDBP	0.5 h post exposure	0.88	0.09 to 1.66	0.03	0.87	0.07 to 1.67	0.03
ΔDBP	20 h post exposure	0.23	−0.55 to 1.00	0.56	0.23	−0.57 to 1.03	0.57
ΔPP	0.5 h post exposure	0.09	−0.72 to 0.91	0.82	0.16	−0.67 to 1.00	0.70
ΔPP	20 h post exposure	0.01	−0.85 to 0.88	0.98	0.16	−0.72 to 1.04	0.72

CI indicates confidence interval; DBP, diastolic blood pressure; PP, pulse pressure; and SBP, systolic blood pressure.

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

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

concentration (Table S1). Therefore, we adjusted for the total exposure mass concentration to determine whether the observed effects of endotoxin and β-1,3-D-Glucan on BP were partially because of 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-minute endotoxin exposure was marginally associated with higher urine VEGF 0.5-hour postexposure. 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 nonsignificantly associated with 3.61 pg/mL increase in urine VEGF at 20-hour postexposure (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 postexposure urine VEGF.

Modification of Endotoxin/β-1,3-D-Glucan Association With BP by VEGF

The association between 130-minute CAP endotoxin exposure and immediate postexposure 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-hour postexposure VEGF was lower than preexposure VEGF and those with a smaller amount of elevation in VEGF at 0.5-hour postexposure (Q1, Q2, Q3); however, the effects were attenuated when there was a large elevation in 0.5-hour postexposure VEGF (Q4). Analysis on β-1,3-D-Glucan did not show notable effect on heterogeneity across ΔVEGF quartiles (Table 3).

Sensitivity Analyses

Exposures to CAP endotoxin and β-1,3-D-Glucan were measured by 2 laboratories; therefore, we performed analysis, including only the data from one laboratory that handled ≈70% of the samples to examine the robustness of our findings. This adjustment only resulted in minor changes in the conclusion, most likely because of compromised statistical power (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 (Table S3 and S4). We also examined the correlation matrix among endotoxin/β-1,3-D-Glucan and cocomponents to identify potential confounding variables by first assessing whether cocomponents were associated ($r \geq 0.6$) with either exposure, and none of the 19 cocomponents met this criterion (Table S5).

Discussion

This study on a single-blind, randomized, crossover controlled human exposure trial demonstrated the physiological impact of short-term ambient particle-associated-endotoxin and β-1,3-D-Glucan on BP and furthermore showed that endotoxin

Table 3. Effect Modification by Vascular Endothelial Growth Factor (VEGF) on the Association Between Short-Term (130 minutes) Endotoxin or β-1,3-D-Glucan Exposure and Blood Pressure (BP)

	0.5 h Post Exposure			20 h Post Exposure		
ΔVEGF	Estimate*	95% CI	*P* Value	Estimate*	95% CI	*P* Value
Endotoxin exposure and ΔSBP						
Midpoint of Q1	2.71	0.77–4.65	0.007	1.49	−0.58 to 3.55	0.16
Midpoint of Q2	2.18	0.62–3.74	0.007	1.21	−0.46 to 2.87	0.15
Midpoint of Q3	1.94	0.46–3.42	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	1.75–5.18	0.0001	1.96	0.28–3.63	0.02
Midpoint of Q2	2.64	1.26–4.03	0.0003	1.66	0.31–3.01	0.02
Midpoint of Q3	2.26	0.93–3.59	0.001	1.53	0.23–2.82	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	0.06–2.08	0.04	0.25	−0.75 to 1.24	0.62
Midpoint of Q2	0.94	0.12–1.76	0.03	0.24	−0.58 to 1.05	0.56
Midpoint of Q3	0.88	0.09–1.68	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

Midpoint of Q1, −49.58 pg/mL; midpoint of Q2, −6.99 pg/mL; midpoint of Q3, 12.67 pg/mL; and midpoint of Q4, 69.14 pg/mL. Results were adjusted for season, exposure type, age, body mass index, sex, chamber temperature and relative humidity, filter configuration, sampling location, and analysis laboratory. CI indicates confidence interval; DBP, diastolic blood pressure; Q1, Q2, Q3, and Q4, the 1st, 2nd, 3rd, and 4th quartile; SBP, systolic blood pressure; and ΔVEGF, the postpre VEGF elevation.

*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.

exposure was associated with increased urine VEGF level immediately after the exposure. In addition, novel findings suggested that individuals with negative or small increases in postexposure urine VEGF level are more susceptible to elevated BP after endotoxin exposure compared with those with more dramatic VEGF increases.

PM exposure contributes to cardiovascular morbidity and mortality, especially during acute exposure, as emphasized by the recent American Heart Association 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 2 hours postexposure.[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 proinflammatory endotoxin effects

(ie, 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 after PM exposure.[2,28] Thus, endotoxin and β-1,3-D-Glucan, 2 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]—whereas 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 with 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 because of demographic characteristics. However, it is possible that there are unmeasured characteristic differences in the 2 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 versus 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 proinflammatory 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 after 2-hour 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 after 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 after short-term endotoxin exposure might be a compensatory humoral-vascular response to an acute endothelial injury that attenuates individual susceptibility to postexposure 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 nondifferential (not associated with participants' exposure status) and is expected to bias our result toward 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 caused by gaseous copollutants. In addition, all exposure experiments were conducted at the same time of the day to eliminate confounding because of diurnal variation. Although residual confounding because of 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 because of 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 as a result of informative missingness is unlikely. Although we did not find that any of the elemental cocomponents 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 epidemiological studies to be at higher risk for pollution health effects (eg, children, older adults, and individuals with preexisting cardiovascular disease).

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 physiological effects of CAPs and may help guide in targeted regulation of particles and their sources for health improvement.

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Disclosures

None.

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Novelty and Significance

What Is New?

- For the first time, we investigated the association between endotoxin and β-1,3-D-Glucan—two major biological particulate matter components—and blood pressure in controlled human exposure experiments.

What Is Relevant?

- Our results suggest that (1) an important determinant of the vascular effect of particulate matter is its biological content, which would aid the development of effective targeted risk-reduction strategy; (2). Vascular endothelial growth factor elevation might be a compensatory humoral-vascular response to an acute endothelial injury that attenuates individual susceptibility to postexposure blood pressure increase.

Summary

Short-term exposures to endotoxin and β-1,3-D-Glucan were associated with increased blood pressure in a randomized crossover trial of controlled human exposure to concentrated ambient particles. Postexposure vascular endothelial growth factor elevation after endotoxin exposure attenuates the effect of endotoxin on blood pressure.