ORIGINAL ARTICLE

Particle Depletion Does Not Remediate Acute Effects of Traffic-related Air Pollution and Allergen

A Randomized, Double-Blind Crossover Study

Denise J. Wooding[1], Min Hyung Ryu[1], Anke Hüls[2,3,4], Andrew D. Lee[1], David T. S. Lin[3,4], Christopher F. Rider[1], Agnes C. Y. Yuen[1], and Chris Carlsten[1]

[1]Air Pollution Exposure Laboratory, Division of Respiratory Medicine, Department of Medicine, and [4]Department of Medical Genetics, University of British Columbia, Vancouver, British Columbia, Canada; [2]Department of Human Genetics, Emory University, Atlanta, Georgia; and [3]Centre for Molecular Medicine and Therapeutics, British Columbia Children's Hospital Research Institute, Vancouver, British Columbia, Canada

ORCID ID: 0000-0003-2455-6575 (C.C.).

Abstract

Rationale: Diesel exhaust (DE), an established model of traffic-related air pollution, contributes significantly to the global burden of asthma and may augment the effects of allergen inhalation. Newer diesel particulate-filtering technologies may increase NO_2 emissions, raising questions regarding their effectiveness in reducing harm from associated engine output.

Objectives: To assess the effects of DE and allergen coexposure on lung function, airway responsiveness, and circulating leukocytes, and determine whether DE particle depletion remediates these effects.

Methods: In this randomized, double-blind crossover study, 14 allergen-sensitized participants (9 with airway hyperresponsiveness) underwent inhaled allergen challenge after 2-hour exposures to DE, particle-depleted DE (PDDE), or filtered air. The control condition was inhaled saline after filtered air. Blood sampling and spirometry were performed before and up to 48 hours after exposures. Airway responsiveness was evaluated at 24 hours.

Measurements and Main Results: PDDE plus allergen coexposure impaired lung function more than DE plus allergen, particularly in those genetically at risk. DE plus allergen and PDDE plus allergen each increased airway responsiveness in normally responsive participants. DE plus allergen increased blood neutrophils and was associated with persistent eosinophilia at 48 hours. DE and PDDE each increased total peripheral leukocyte counts in a manner affected by participant genotypes. Changes in peripheral leukocytes correlated with lung function decline.

Conclusions: Coexposure to DE and allergen impaired lung function, which was worse after particle depletion (which increased NO_2). Thus, particulates are not necessarily the sole or main culprit responsible for all harmful effects of DE. Policies and technologies aimed at protecting public health should be scrutinized in that regard.

Clinical trial registered with www.clinicaltrials.gov (NCT02017431).

Keywords: diesel exhaust; asthma; filter; genetic susceptibility

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Correspondence and requests for reprints should be addressed to Chris Carlsten, M.D., M.P.H., 2775 Laurel Street 7th Floor, the Lung Center, Vancouver General Hospital—Gordon and Leslie Diamond Health Care Centre, Vancouver, BC, V5Z 1M9 Canada. E-mail: carlsten@mail.ubc.ca.

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At a Glance Commentary

Scientific Knowledge on the Subject: Health benefits of particulate-reducing technologies have been largely untested.

What This Study Adds to the Field: Particle depletion of diesel exhaust increased NO_2 and exacerbated allergen-induced loss of lung function, particularly in those genetically at risk.

Air pollution was responsible for an estimated 6.5 million global deaths in 2015 (1); in some countries, half of all mortality relating to air pollution can be attributed to motorized traffic (2). Traffic-related air pollution (TRAP) exposure has been strongly linked to airways disease, including asthma incidence, hospitalization, and related symptoms, particularly in children (2–4). However, the mechanism(s) through which TRAP may lead to the development or exacerbation of asthma are not well understood.

Previous acute human exposure studies have demonstrated that short-term exposure to TRAP can impair lung function, induce systemic inflammation, and, in subjects with asthma, increase airway hyperresponsiveness (AHR) (5–9). Recent work suggests that the effects of coexposure to diesel exhaust (DE) and allergen on nasal/pulmonary inflammatory markers may be greater than the added effects of either one alone (10, 11), a synergy that is important to understanding the true health effects of this nearly ubiquitous pollutant. Our group previously revealed that coexposure to inhaled DE, along with allergen instilled during lung segmental allergen challenge, augmented allergic inflammation in the lower airways relative to allergen alone (10). Furthermore, such effects were more pronounced in genetically "at-risk" individuals, who were GST (glutathione-*S*-transferase) T1 null (10). Similar augmented coexposure effects have been demonstrated in a nasal challenge model (11). The novelty of this article reflects our motivation, in this context, to understand the following unknowns: the effects of particle depletion on DE's augmentation of allergenic effects, the potential role of genetic susceptibility in modifying this response, and the role of a more realistic inhaled (as opposed to instilled) allergen challenge model therein. We hypothesized that particle depletion would protect against the harmful effects of DE and allergen coexposure, and that the effects of coexposures would be augmented in genetically at-risk individuals.

It is worth noting, therefore, that a number of diesel engine technologies have emerged in an effort to curtail the growing public health impact of TRAP (12). Among them, diesel particulate filters (DPFs) generally remove approximately 90% of the particulate fraction of DE emissions, but often increase NO_2 emissions (13). By 2011, an estimated 250,000 on-road, heavy-duty vehicles were retrofitted with DPFs worldwide, and since 2000, millions of new diesel passenger cars in Europe have been produced with DPFs (14). In Canada and the United States, all heavy-duty diesel highway engines produced since 2007 were equipped with DPFs in an effort to meet increasingly stringent emissions standards set by Environment Canada and the U.S. Environmental Protection Agency (14). Although the particulate emissions from DPF-fitted engines are drastically reduced, their effectiveness in protecting health in the context of TRAP and allergen coexposure is not well understood. Previous controlled human exposure studies achieving approximately 50% particle depletion have demonstrated that symptoms, lung function deficits, and pulmonary inflammation induced by DE were not attenuated by particle depletion (8, 9). However, the effectiveness of particle depletion has not been tested in the context of DE and allergen coexposures, using technology that more effectively reduces particulate matter (at the cost of higher gaseous output). Accordingly, we aimed to address two particular concerns: *1*) to directly assess the effects of DE and allergen coexposure on lung function, airway responsiveness, and circulating leukocyte counts, by simulating real-world conditions with inhaled DE and inhaled allergen challenge models; *2*) to determine the efficacy of particle depletion in protecting against these harmful effects. Some of the results of these studies have been previously reported in the form of an abstract (15).

Methods

Study Design

A total of 14 allergen-sensitized individuals participated in this randomized, double-blind, controlled human exposure crossover study taking place between April 2013 and April 2017 (Clinical Trials no. NCT02017431). All participants gave written, informed consent to the study protocol, which was approved by the University of British Columbia Research Ethics Board (H11-01831), Vancouver. The sample size was based on previous studies detecting a change in blood eosinophil counts after allergen challenge with 12 subjects (16), and blood neutrophil counts after DE exposure with 15 subjects (5). Exposures were performed at the Air Pollution Exposure Laboratory at Vancouver General Hospital. Each individual was exposed to all of the four coexposure conditions in random order, each separated by a 4-week washout period: filtered air plus 0.9% saline (FA-S; the negative control); FA plus allergen (FA-A); DE diluted to 300 $\mu g/m^3$ of particulate matter $\leqslant$2.5 μm in aerodynamic diameter ($PM_{2.5}$) + allergen (DE-A); and particle-depleted DE + allergen (PDDE-A) (Figure 1). Participants stopped bronchodilator use at least 48 hours before study visits, and exposures were not performed on days when participants reported any change in bronchodilator use in the days before a visit or exhibited cold or flu symptoms (Common Cold Questionnaire; *see* detailed Methods in the online supplement).

Participants

We recruited nonsmoking adults, sensitized to at least one of birch, grass, or house dust mite. AHR was assessed at the first screening visit by a methacholine challenge using the 2-minute tidal breathing technique (17). The provocative concentration of methacholine eliciting a 20% drop in FEV_1 (PC_{20}) during screening was used to define participants as either hyperresponsive ($PC_{20} \leqslant 8$ mg/ml) or normally responsive ($PC_{20} > 8$ mg/ml). Six hyperresponsive and two normally responsive participants reported that they had previously received an asthma diagnosis from a physician. Due to this discordance, groups are classified herein based on our measured airway responsiveness, rather than asthma diagnosis. Participant characteristics are detailed in Table 1.

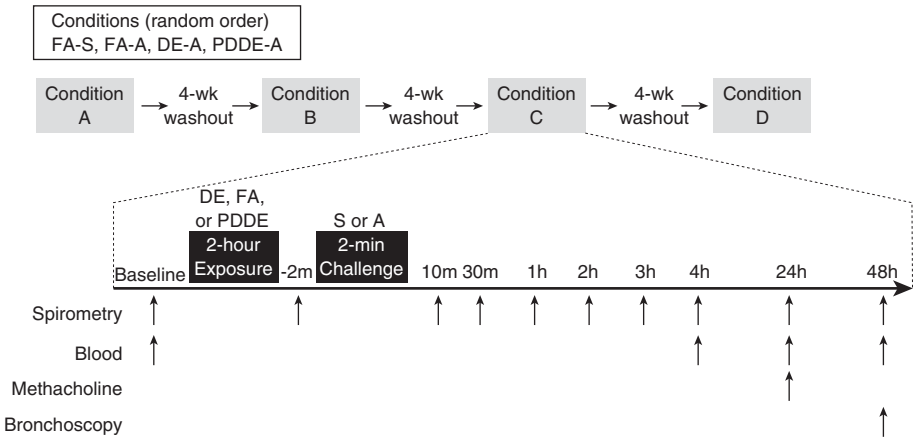

Figure 1. Randomized, double-blind crossover study design. Subsequent to meeting study inclusion criteria in two prior screening visits, consenting participants visited the laboratory for four 3-day visits, each over 48 hours. There was a minimum 4-week washout period between each visit. On Day 1 of each visit, baseline blood samples were collected and spirometry was performed before a 2-hour exposure to filtered air (FA), diesel exhaust (DE; at 300 $\mu g/m^3$ particulate matter ≤ 2.5 μm in aerodynamic diameter), or particle-depleted DE (PDDE). Spirometry was reassessed immediately after exposure, before 2 minutes of inhaled saline (S) or allergen challenge (A) was administered. Spirometry and blood samples were collected through 48 hours after exposure. Airway responsiveness (provocative concentration of methacholine eliciting a 20% drop in FEV_1 [PC_{20}]) was assessed at 24 hours after exposure using a methacholine challenge after the 2-minute tidal breathing protocol.

Exposures

Exposures (FA, DE, and PDDE) were 2 hours in duration and contained trivial levels of endotoxin (18) (Table 2). For PDDE, particulates were removed by high-efficiency particulate absolute filtration and electrostatic precipitation, generating a representation of newer DPF technology that creates PM-reduced, but NO_2-enriched, exhaust (*see* Figure E1 in the online supplement) (19).

Inhaled Allergen Challenge

One hour after exposure, a 2-minute inhaled allergen challenge was performed using an allergen PC_{20} dose that was determined at screening based on PC_{20} and skin prick wheal size (20).

Lung Function, AHR, and Blood Leukocytes

Spirometry was performed in accordance with the American Thoracic Society's guidelines (21) before and through 48 hours after exposure, and airway responsiveness was reassessed by methacholine challenge 24 hours after each exposure (17). Circulating leukocyte counts were measured before and after exposures; 13 participants performed all 3 days of each of the 4 exposure conditions, and 1 participant voluntarily withdrew from the study after the third visit due to scheduling conflicts.

Genotyping and Genetic Risk Score

Sixteen null alleles, micro insertion/deletion sites or SNPs were selected as targets to construct a genetic risk score for each participant who completed the study (Table E1) (22–25). These allelic variants were previously suggested to modulate the response to air pollution (23–25). Each individual was assigned an unweighted genetic risk score, which is defined as the unweighted sum of the number of SNP risk alleles (or null for GSTT1 or GSTM1). Details on the genotyping procedure are provided in Tables E2 and E3 and the METHODS in the online supplement.

Table 1. Participant Characteristics

Participant	Age (*yr*)	FEV_1% Predicted	FEV_1/FVC	Genetic Risk Score	PC_{20} (*mg/ml*)	Sex	Allergen (Concentration)
1	30	108	0.89	15	>128	M	HDM (1/32)
2	28	105	0.87	12	>128	F	HDM (1/32)
3	46	97	0.78	13	>128	F	Grass (1/256)
4	50	122	0.84	N/A*	>128	F	Birch (1/64)
5	23	105	0.87	9	>128	M	Grass (1/1,024)
6	23	102	0.69	6	0.5	M	Grass (1/66,538)
7	32	111	0.76	5	5.9	F	HDM (1/128)
8	28	104	0.78	7	2.6	M	HDM (1/512)
9	33	86	0.73	9	0.8	M	Grass (1/4,096)
10	24	100	0.74	7	0.9	F	HDM (1/16,384)
11	25	107	0.84	12	6.8	M	HDM (1/512)
12	23	84	0.79	12	1.6	F	Grass (1/256)
13	44	114	0.82	6	6.9	F	HDM (1/4)
14	28	123	0.87	10	3.5	M	Birch (1/8)
Summary†	31 ± 9	105 ± 11	0.81 ± 0.06	8 ± 3	5 normally responsive 9 hyperresponsive	7 M 7 F	7 HDM 5 grass 2 birch

Definition of abbreviations: HDM = house dust mite; N/A = not available; PC_{20} = provocative concentration of methacholine eliciting a 20% drop in FEV_1. Genetic risk score is the unweighted sum of the number of SNP minor alleles (or null for GSTT1 or GSTM1) of 14 selected SNPs.
*Genotyping was not performed for this participant.
†Mean ± SD for age, FEV_1% predicted, FEV_1/FVC, and genetic risk score.

Table 2. Exposure Characteristics

	CO (*ppm*)	CO_2 (*ppm*)	NO_2 (*ppb*)	NO (*ppb*)	NO_x (*ppb*)	$PM_{2.5}$ ($\mu g/m^3$)	TVOCs (*ppb*)
FA	3 ± 2.1	696.8 ± 56.2	2.8 ± 1.7	22 ± 14.3	24.8 ± 14.8	2.6 ± 1.8	300.7 ± 29.2
DE	14 ± 3.4	2,044.1 ± 202.4	52.5 ± 40.1	2,770.1 ± 583	2,822.5 ± 569.6	292.2 ± 24.2	1,932.3 ± 145.4
PDDE	15.2 ± 8.3	2,101.6 ± 220.2	150.3 ± 86.2	2,555.2 ± 449.4	2,705.6 ± 447.6	18.9 ± 8.5	1,750.9 ± 150.6

Definition of abbreviations: CO = carbon monoxide; CO_2 = carbon dioxide; DE = diesel exhaust; FA = filtered air; NO = nitric oxide; NO_2 = nitrogen dioxide; NO_x = nitric oxides; PDDE = particle-depleted diesel exhaust; $PM_{2.5}$ = particulate matter ≤2.5 μm in aerodynamic diameter; TVOCs = total volatile organic compounds.

Statistical Analyses

Outcomes presented herein for this clinical trial are not the primary or secondary outcomes registered, which will be presented elsewhere. Effects of exposures were assessed using linear mixed effects models (nlme package version 3.1-131) in R (version 3.4.3). Initially, conditions (FA-S, FA-A, DE-A, or PDDE-A) were used as the fixed effect, and participant identification as the random effect. Previous work showed that individuals with asthma have increased AHR after exposure to DE or NO_2 (7, 26, 27). Thus, it was hypothesized that hyperresponsive individuals would have a greater reduction in PC_{20} after coexposures. Therefore, a second model was employed where condition-by-group (normally responsive vs. hyperresponsive) interaction was the fixed effect. A third model, with condition-by-genetic risk score interaction as the fixed effect, was used to assess the potential role of genetic susceptibility in modulating responses. *P* values less than 0.05 were considered statistically significant. The potential for order effects were not statistically assessed, as there were 13 unique orders in which the exposures were delivered in our study. Therefore, the likelihood of a carryover effect producing a false-positive result is extremely low. Nonetheless, 18 days was previously shown to be sufficient to avoid carryover in immunological endpoints from a DE particle challenge (28). The area under the FEV_1 curve (AUC) was calculated across time in minutes, from −2 minutes to 4 hours (representative of sensitive postexposure period), and 48-hour (representative of full follow-up period) time points shown in Figure 1. Blood cell counts that were significantly modified by exposure were tested for their association with 30-minute FEV_1, as well as the 48-hour AUC, by repeated measures correlation using the rmcorr package (version 0.3.0).

Results

Effective Particle Depletion Decreased Total Volatile Organic Compounds but Increased NO_2 Levels

$PM_{2.5}$ was effectively depleted, on average, 94% in the PDDE (18.9 $\mu g/m^3$) condition relative to DE (292.2 $\mu g/m^3$) (Figure 2). In the gaseous fraction, particle depletion led to a decrease in total volatile organic compounds (TVOCs) and an increase in NO_2. The geometric mean aerodynamic diameter of particles in the mixture was significantly greater in DE (84 nm; 95% confidence interval [CI] = 77–91 nm, $P < 0.0001$) and PDDE (75 nm; 95% CI = 66–83 nm, $P < 0.05$) than FA (63 nm; 95% CI = 58–68 nm), with no difference between DE and PDDE. As noted in the following mean differences, other measured components of the exhaust were not statistically different in DE relative to PDDE: CO (0.8; 95% CI = −1.7 to 3.2); CO_2 (−58; 95% CI = −208 to 93); NO (215; 95% CI = −149 to 579); and NO_x (117; −242 to 476). Consistent with our previously published exposure characteristics (18), endotoxin levels in the current study were below the threshold limit of detection (0.5 EU/m^3).

Particle Depletion Exacerbated Acute Airflow Impairment Induced by DE and Allergen Coexposure, Particularly in Those Genetically at Risk

There was no effect of DE or PDDE on lung function before delivering the allergen challenge (Figure 3). As expected, lung function was significantly reduced in all three conditions that included allergen (FA-A, DE-A, and PDDE-A) relative to FA-S. At 30 minutes after exposure, the typical peak of response to allergen-induced lung function decline (29), PDDE-A elicited a 7.5% greater impairment in FEV_1 than DE-A (95% CI = −0.07% to −14.8%, $P = 0.047$), suggesting that particle depletion exerted a deleterious, rather than protective, effect on lung function (Figure E2). After Bonferroni correction for multiple comparisons, which is conservative in this context of nonindependent measures and modest risks of type II error (30), there was no difference between DE-A and PDDE-A. However, a *post hoc* analysis to estimate the isolated effect of PDDE versus DE showed that, after normalization by subtracting FA-A, the 30-minute change in FEV_1 associated with PDDE-A was greater than DE-A (mean difference of PDDE-A minus FA-A vs. DE-A minus FA-A = 8.3%; 95% CI = −16.3 to −0.25, $P = 0.05$). Furthermore, the effect of condition on FEV_1 was significantly modified by genetic risk score only in the PDDE-A condition; individuals with higher genetic risk score had a greater decrease in FEV_1 at 30 minutes, particularly with PDDE-A ($P = 0.008$ for condition-by-genetic risk score interaction). FEV_1 resolved to the point of nonsignificance relative to baseline by 48 hours across all conditions. The 4-hour AUC was significantly increased by FA-A (2.0×10^3 L · min, 95% CI = 0.8–3.2, $P = 0.001$), DE-A ($+1.6 \times 10^3$ L · min, 95% CI = 0.4–2.7, $P = 0.011$), and PDDE-A ($+2.1 \times 10^3$ L · min, 95% CI = 1.0–3.3, $P = 0.001$), and the 48-hour AUC was increased by FA-A ($+2.4 \times 10^4$ L · min, 95% CI = 1.1–3.7, $P = 0.001$), DE-A ($+1.9 \times 10^4$ L · min, 95% CI = 0.6–3.2, $P = 0.007$), and PDDE-A ($+2.2 \times 10^4$ L · min, 95% CI = 0.9–3.5, $P = 0.002$) relative to FA-S, with no significant differences between these three conditions. A significant effect of genetic risk score on AUC is detailed in the online supplement.

Coexposure to Allergen and DE, with and without Particulates, Significantly Increased Airway Responsiveness in Normally Responsive Participants

Both DE-A and PDDE-A significantly augmented airway responsiveness only in

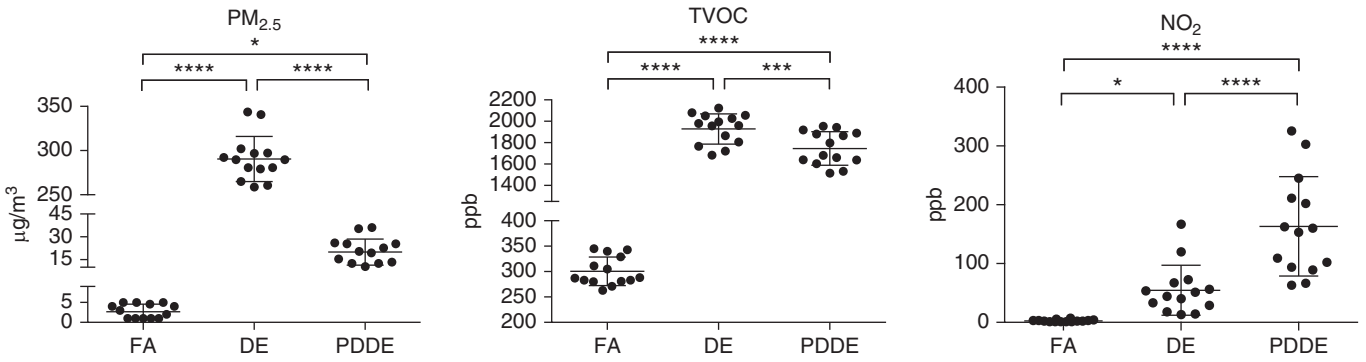

Figure 2. Comparison of filtered air (FA), diesel exhaust (DE), and particle-depleted diesel exhaust (PDDE). Particulate matter ≤2.5 μm in aerodynamic diameter ($PM_{2.5}$) was depleted, on average, 94% in PDDE, whereas total volatile organic compounds (TVOCs) decreased 9.5% and nitrogen dioxide (NO_2) increased, on average, 350%. Each point represents one exposure; lines represent mean ± SD and differences were quantified using one-way ANOVA with Tukey's *post hoc* test. $^{*}P < 0.05$, $^{***}P < 0.001$, and $^{****}P < 0.0001$.

the normally responsive group (mean decrease in $PC_{20} = -13.55$ mg/ml after DE-A, $P = 0.035$; −13.2 mg/ml after PDDE-A, $P = 0.027$ [i.e., 1.8 and 2.5 doubling doses, respectively]; Figure 4). There was no significant difference between DE-A and PDDE-A, indicating that particle depletion did not protect against the effects of coexposure on AHR. Alternatively, there was no main effect of exposure on airway responsiveness (across all participants) and, contrary to our hypothesis, no effect in the hyperresponsive participants.

DE and Allergen Coexposure Led to Blood Neutrophilia and Prolonged Eosinophilia

Effects of exposure on systemic WBC counts are summarized in Table 3. Only DE-A and PDDE-A increased total WBC counts at 24 hours (Table 3 and Figure 5). Only DE-A increased neutrophil counts at 24 hours, and was associated with persistent blood eosinophilia at 48 hours. Monocyte counts were increased at all time points, and eosinophils at 24 hours, after FA-A, DE-A, and PDDE-A exposure. Changes in cell counts were, almost universally, negatively correlated with changes in FEV_1 at 30 minutes and in the FEV_1 AUC (Table 4). Significant effect modification by genetic risk score is detailed in the online supplement.

Discussion

Particulate-filtering technologies are attractive for their desired potential to mitigate the harmful effects of particulate air pollution, and are already promoted by a number of environmental regulatory agencies (13, 14). However, the current study demonstrates, for the first time, that exposure to PDDE, in the presence of allergen, may actually exacerbate some adverse effects of DE on biological and clinical endpoints compared with typical DE. Our data suggest that this deleterious effect of particle depletion may be attributable to differences in the gaseous fraction of PDDE. Of the measured gaseous components of DE (TVOCs, CO, CO_2, NO, and NO_2), the only differences between DE and PDDE in our study were that PDDE contained lower TVOCs and higher NO_2 levels in the emissions, pointing to NO_2 as a potentially important player in these responses. This is particularly important, as recent studies show diesel truck engines trending toward higher NO_x when assessed in real-world conditions (31).

Perhaps most noteworthy in our results is that PDDE-A impaired FEV_1 to a larger extent than DE-A. Although the cause of this worsening is unclear, the increased NO_2 in PDDE seems a plausible contributor. Previous work has demonstrated that acute NO_2 monoexposure can increase airway resistance (26), and NO_2 can also augment airway responsiveness to an allergen challenge (32). Furthermore, indoor NO_2 exposures are associated with airflow obstruction in children with atopy (33). It has been previously determined that a change of 0.23 L in FEV_1 could be considered clinically important in the context of asthma (34). In the current study, PDDE-A reduced FEV_1 7.5% more

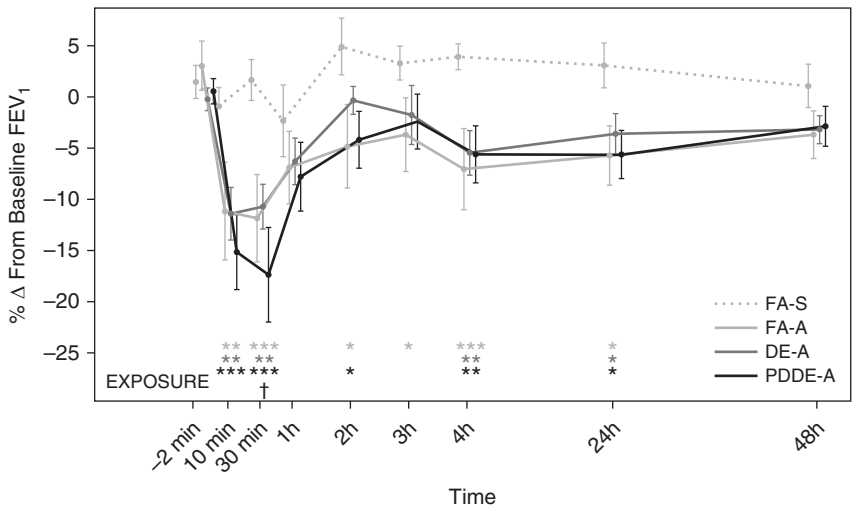

Figure 3. Effect of exposures on FEV_1. Values are expressed as percentage change from same-condition baseline measurements. Lines represent means of all participants ($n = 14$), and error bars represent SE. Significance relative to FA-S: $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$. Significance relative to DE-A: $^{\dagger}P < 0.05$. For definition of abbreviations, *see* Figure 1.

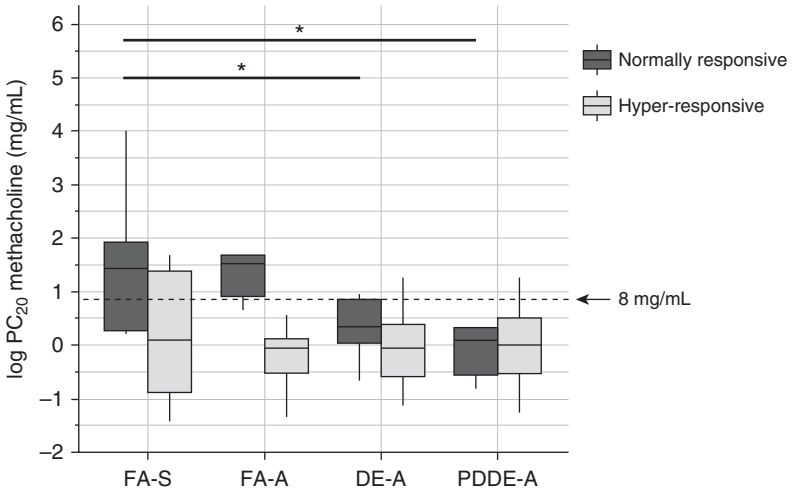

Figure 4. Effect of exposure on airway hyperresponsiveness. The provocative concentration of methacholine eliciting a 20% drop in FEV_1 (PC_{20}) was measured 24 hours after exposure. Significance bars represent differences induced by exposure, which existed only in the normally responsive group. *$P < 0.05$. Boxes show mean with 25th and 75th percentiles; whiskers extend to the largest and smallest value at most 1.5 × interquartile range from box edge. The dashed line represents 8 mg/ml methacholine (for reference as a "threshold" for hyperresponsiveness). For definition of abbreviations, *see* Figure 1.

than DE-A on average, or 0.39 L in absolute terms. Therefore, the magnitude of the worsening effect of PDDE-A is, although transient, clinically relevant (35). These findings are in contrast with a previous study, which demonstrated lower levels of thrombus formation and improved response to vasodilators after exposure to particle-depleted exhaust relative to regular DE in healthy male volunteers (36). Differences between this and the current study, including subject population, endpoints, and the use of coexposures, highlights the importance of delineating the effects of particle traps across a range of exposure conditions and biological endpoints.

The method of particle depletion in the current study, electrostatic precipitation, oxidizes NO, which increases levels of NO_2 in the exhaust. Although, in one sense, it is a limitation that we were not able to assess the isolated effects of particle depletion (with all gaseous components remaining equal), our exposure characteristics may in fact be more reflective of real-world DPFs and diesel oxidation catalysts, which tend to suffer from the same trade off (reduced PM and increased NO_2) (37). It should be noted that other after-treatment methods exist, such as selective catalytic reduction technologies, which are designed to reduce NO_x emissions. However, these only recently began to be fitted on new vehicles, still face durability challenges, have suffered from efforts that bypass their effectiveness (38), and have highly variable performance that is dependent on operating conditions (39). DPF technologies have already contributed to a measurable change in roadside pollutants: between 2002 and 2004, the roadside ratio of NO_2:NO_x in London approximately doubled, coinciding

Table 3. Effect of Exposures on Blood Cell Counts and Effect Modification by Genetic Risk Score

			FA-A		DE-A		PDDE-A	
	Time (*h*)	FA-S Mean	Effect (95% CI)	*P* Value	Effect (95% CI)	*P* Value	Effect (95% CI)	*P* Value
WBC	24	−0.21	0.57 (−0.24 to 1.38)	0.18	0.90 (0.11–1.69)	**0.03**	1.10 (0.28 to 1.93)	**0.01**
	48	−0.45	0.30 (−0.42 to 1.03)	0.42	0.64 (−0.07 to 1.36)	0.09	0.64 (−0.09 to 1.37)	0.10
Neutrophils	24	0.03	0.38 (−0.32 to 1.08)	0.29	0.72 (0.05–1.40)	**0.04**	0.67 (−0.03 to 1.38)	0.07
	48	−0.36	0.29 (−0.33 to 0.91)	0.37	0.21 (−0.40 to 0.83)	0.50	0.56 (−0.07 to 1.19)	0.09
Lymphocytes	24	−0.07	0.08 (−0.21 to 0.38)	0.59	−0.06 (−0.34 to 0.22)	0.67	0.07 (−0.23 to 0.37)	0.64
	48	0.01	−0.13 (−0.37 to 0.12)	0.32	−0.19 (−0.42 to 0.05)	0.14	−0.18 (−0.42 to 0.07)	0.16
Monocytes	24	−1.18	1.28 (0.67–2.30)	**0.001***	1.19 (0.41 to 1.97)	**0.005***	1.22 (0.41 to 2.04)	**0.006***
	48	−1.22	1.18 (0.47–1.91)	**0.003***	1.23 (0.53 to 1.94)	**0.002***	1.24 (0.51 to 1.96)	**0.002***
Eosinophils	24	−0.03	0.14 (0.04–0.24)	**0.01**	0.17 (0.07 to 0.27)	**0.002**	0.23 (0.13 to 0.33)	**0.0001**
	48	0.01	0.08 (−0.07 to 0.23)	0.31	0.17 (0.02 to 0.32)	**0.03**	0.12 (−0.03 to 0.27)	0.13
Basophils	24	−0.006	−0.002 (−0.035 to 0.031)	0.896	0.006 (−0.025 to 0.038)	0.71	0.014 (−0.019 to 0.047)	0.41
	48	−0.008	−0.001 (−0.043 to 0.042)	0.98	0.008 (−0.034 to 0.049)	0.72	0.024 (−0.018 to 0.067)	0.27

Definition of abbreviations: CI = confidence interval; DE-A = diesel exhaust + allergen; FA-A = filtered air + allergen; FA-S = filtered air + saline; PDDE-A = particle-depleted diesel exhaust + allergen; WBC = white blood cells.
Cell count values are expressed as unit change (cells × 10^9/L). FA-S mean represents the mean change in counts after FA-S relative to before exposure. Effect columns represent mean change across the exposure relative to FA-S mean. Significant exposure effects ($P < 0.05$) are bold.
*Exposure effects that were significantly modified by genetic risk score, such that a higher genetic risk score was associated with a greater increase in cell counts after exposure ($P < 0.05$).

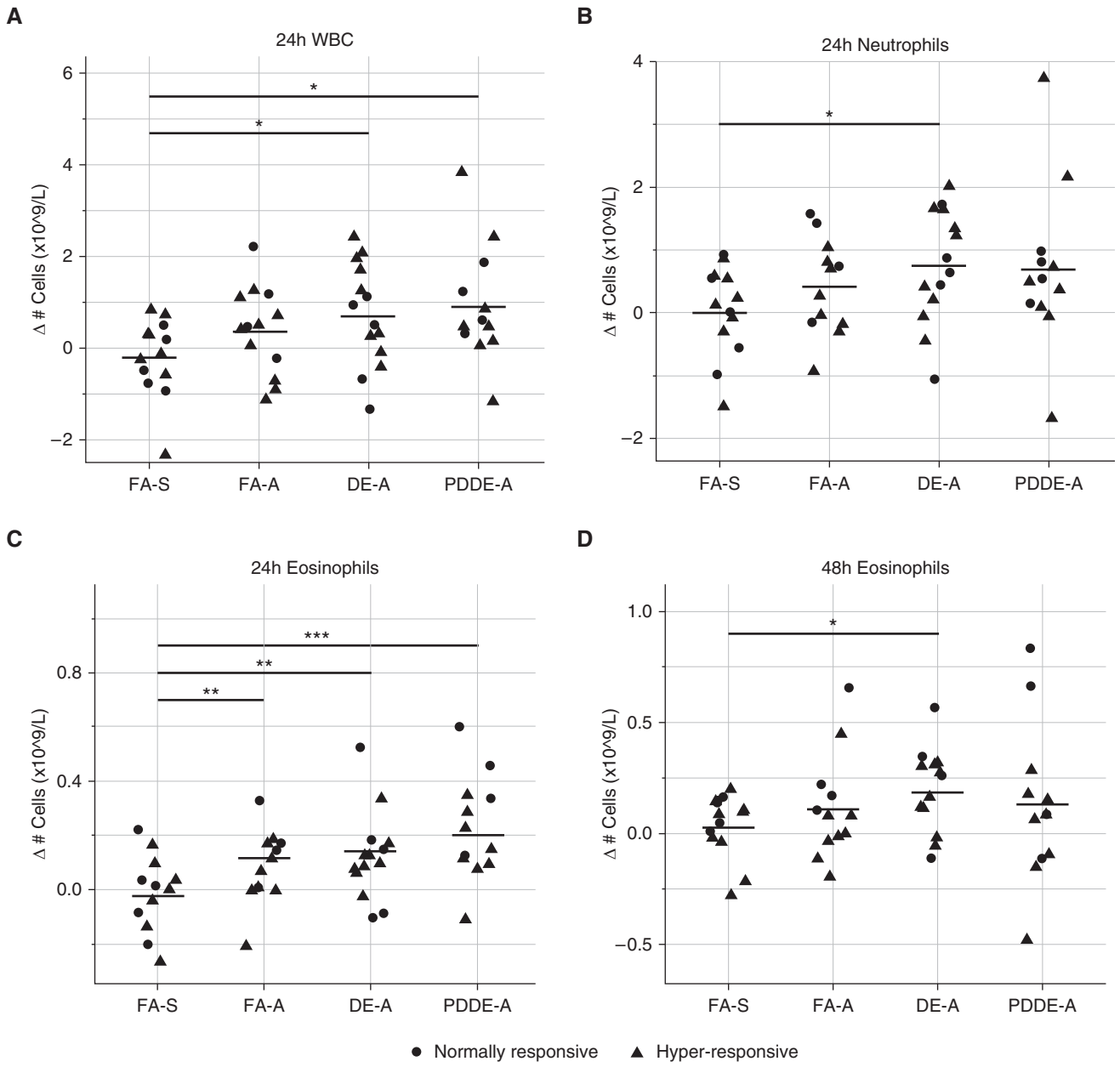

Figure 5. Effect of exposure on blood inflammatory cell counts. Each point represents the change in blood cell counts from baseline to (*A–C*) 24 hours and (*D*) 48 hours after exposure for one participant. Significance is denoted by $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$. FA-A = filtered air + allergen; FA-S = filtered air + saline; DE-A = diesel exhaust + allergen; PDDE-A = particle-depleted diesel exhaust + allergen; WBC = white blood cells.

with the London bus fleet being fitted with DPFs (13). Future work would benefit from assessing the longer-term impact of repeated exposures under similar coexposure conditions to our current study.

This study is the first to directly observe an increase in airway responsiveness, in individuals normally responsive at baseline, in the context of acute air pollution exposure (although an increase in airway resistance has been shown previously in response to DE [40], that observation may or may not have been due to changes in airway responsiveness, which was unassessed in that study, and was also not in the context of allergen coexposure). Specifically, after DE-A or PDDE-A exposure, PC_{20} was significantly reduced in those who had normal PC_{20} values at baseline, despite allergen alone having no effect. Although these phenomena are presumed transient, and thus not truly representative of *de novo* disease, coexposures should be duly considered (41) in future observational efforts to understand new-onset AHR and/or asthma development. Previously, both DE and NO_2 were shown to increase airway responsiveness in some subjects with asthma (7, 26). The effect in our study was not mitigated by particle depletion, suggesting an important role for the gaseous fraction of DE. It is unclear why individuals from the hyperresponsive group

Table 4. Repeated Measures Correlation between Change in Blood Cell Counts and Change in FEV_1 across Exposures

Cell	Time (*h*)	30-min FEV_1 % Change			AUC FEV_1 % Change		
		r	95% CI	*P* Value	*r*	95% CI	*P* Value
Monocytes	24	−0.454	−0.12 to −0.70	**0.008**	−0.42	−0.66 to −0.11	**0.009**
	48	−0.53	−0.21 to −0.75	**0.002**	−0.39	−0.64 to −0.06	**0.019**
Eosinophils	24	−0.573	−0.27 to −0.77	**0.0005**	−0.57	−0.76 to −0.29	**0.0002**
	48	−0.394	−0.03 to −0.66	**0.026**	−0.47	−0.70 to −0.16	**0.003**
WBC	24	−0.432	−0.10 to −0.68	**0.011**	−0.29	−0.57 to 0.04	0.076
Neutrophils	24	−0.396	−0.05 to −0.66	**0.022**	−0.24	−0.53 to 0.10	0.154

Definition of abbreviations: AUC = area under the FEV_1 percentage change curve across the 48-hour follow-up period; CI = confidence interval; WBC = white blood cells.

Blood cell counts that were significantly altered by exposure (Table 3) were assessed for their correlation with changes in airflow. Columns labeled "*r*" reflect correlation coefficient between change in blood cell counts relative to before exposure, and (in the first instance) percentage change in FEV_1 at 30 minutes relative to before exposure and (in the second instance) area under the FEV_1 percentage change curve, across the entire 48-hour follow-up period (*see* Figure 3). Statistically significant values ($P < 0.05$) are denoted in bold.

did not see an increase in airway responsiveness, as has been suggested previously (7, 26). Although speculative, we suggest that increased airway responsiveness is more likely in the context of allergen coexposure than with DE alone, as our previous work also demonstrated a trend in this direction (42) (with only a segmental allergen challenge, expected to be less potent in driving this effect than would be inhaled whole-lung allergen, as in our current study).

As noted previously, we earlier tested the effects of DE and allergen coexposure using a segmental allergen challenge (10), which precluded us from delineating systemic effects. Accordingly, the current study is the first to examine the effects of DE-A and PDDE-A coexposures on circulating leukocytes. In the current study, blood eosinophilia persisted for up to at least 48 hours only after DE-A exposure. This may be a consequence of particle deposition prolonging the effective exposure seen by the lung (43). This is consistent with previous work demonstrating that ultrafine particles, including the average particle diameter of 100 nm in the current study, have high levels of deposition, may evade phagocytosis, and may penetrate into interstitial sites and even the circulation (43).

In addition, only DE-A significantly increased neutrophil counts, whereas there was a strong trend for PDDE-A ($P = 0.07$), and only these coexposures, but not FA-A, significantly increased total WBC count. Previous controlled human studies of exposure to DE alone found effects on differential blood cell counts to be modest (i.e., neutrophils alone being affected at 6 h) or absent (5, 44). However, DE plus ozone was previously shown to induce peripheral monocyte, lymphocyte, and neutrophils effects, the latter two of which persisted for up to 22 hours, highlighting the importance of coexposures in eliciting potentially greater responses (45). The negative correlation between blood cell counts and FEV_1 suggests that circulating leukocytes may play a meaningful role in eliciting lung function decrements in the context of these exposures. Elevated peripheral WBC counts, which were observed after DE-A and PDDE-A, are also an independent predictor of all-cause mortality at the epidemiological level (46). Nonetheless, there was no difference between DE-A and PDDE-A exposure on blood leukocyte counts, indicating that, contrary to the hypothesis, particle depletion did not demonstrate a protective effect therein, suggesting that DE-A was no worse than PDDE-A in this regard. However, we were unable to perform an appropriate noninferiority analysis due to the lack of established minimally important clinical difference for these endpoints. Furthermore, our study may be underpowered for such an analysis.

Finally, it is worth noting that long-term exposure to $PM_{2.5}$ increases risk of cardiovascular disease and mortality steeply at low concentrations, but subsequently responses tend to plateau (47). Dose–response relationships with regard to short-term settings are less well understood, but it is notable that, in our acute exposure, $PM_{2.5}$ was reduced from 290 (±25) $\mu g/m^3$ in DE to 20 (±9) $\mu g/m^3$ in PDDE. If these remaining low levels are near the plateau of the dose–response curve in our acute setting, it could explain why overall DE-A and PDDE-A were more similar than different. However, this is speculation until dose–response relationships are better defined in the context of acute exposures.

In conclusion, we showed that DE and allergen coexposures decreased FEV_1 and increased peripheral WBC counts. These adverse effects persisted even after particle depletion, which suggests that some diesel particulate–filtering technologies may not protect against the harmful effects of DE, particularly in the context of allergen coexposure. Future work should aim to delineate the effects of longer-term exposures to TRAP and allergen, and the potential role of peripheral inflammation in development of asthma as it relates to these coexposures. Furthermore, using genetic risk scores, we identified susceptible subgroups defined by their number of oxidative stress–associated risk alleles. Future work should continue to assess who is most at risk, which components of DE are most harmful, and what technologies, policies, and practices could be employed to reduce TRAP emissions and, hence, the substantial harm to public health attributable to air pollution exposure. ■

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