Inhalation of allergen and diesel exhaust increases urinary eicosanoids associated with impaired lung function and airway hyperresponsiveness: a randomized, double-blinded, human crossover study

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ABSTRACT

Background. Eicosanoids are potent regulators of homeostasis and inflammation that play an important role in asthma pathophysiology. In a controlled human exposure study, we showed that coexposure to allergen and diesel exhaust (DE) led to eosinophilic inflammation, impaired airflow, and increased airway responsiveness. Eicosanoids may mediate the mechanism by which these exposures impair lung function.

Methods. We conducted a randomized, double-blinded, four-arm crossover study. Fourteen allergen-sensitized participants were exposed to four conditions: filtered air and saline (FA-S; negative control); filtered air and allergen (FA-A; allergen alone); DE and allergen (DE-A; coexposure); and particle-depleted DE and allergen (PDDE-A; coexposure with minimal particles). Quantitative metabolic profiling of urinary eicosanoids was performed using LC-MS/MS.

Results. Allergen inhalation increased urinary eicosanoids. The prostacyclin metabolite 2,3-dinor-6-keto-$PGF_{1\alpha}$ increased with DE-A, but particle depletion (PDDE-A) suppressed this pathway. Baseline airway hyperresponsiveness modified the allergen-induced increase in prostaglandin D_2 metabolites (tetranor PGDM and 2,3-dinor-11β-$PGF_{2\alpha}$) such that normally responsive individuals showed a greater allergen-induced increase of the two metabolites. Genetic risk scores (GRS) modified the effect of DE-A; those individuals with a high GRS demonstrated a greater increase in isoprostane metabolites following DE-A. Increased urinary leukotriene E_4 and tetranor PGDM correlated with increased airway responsiveness, while increased tetranor PGDM also correlated with decline in FEV_1.

Conclusions. Impaired airflow and increased airway responsiveness upon exposure to DE-A may be attributable to increased levels of leukotrienes and prostaglandins. Variants in genes known to mediate response to pollution appear to modulate these eicosanoid-mediated physiological responses known to contribute to asthma pathophysiology.

Keywords: air pollution, allergen inhalation, diesel exhaust, eicosanoids, gene-environment interaction

INTRODUCTION

Eicosanoids are bioactive lipid mediators derived from arachidonic acid. They exert their effects at the tissue level to maintain homeostasis and regulate the initiation, amplification, and resolution of inflammatory processes.[1] During the inflammatory response, eicosanoids are excreted by structural and inflammatory cells in the local milieu. Eicosanoids are then rapidly metabolized, cleared by the vasculature, and excreted in the urine.[2] Monitoring the excretion level of eicosanoids in urine is accordingly a valuable non-invasive tool to profile and quantify pathophysiological processes in the lung,[3] including the body's immune response to environmental stimuli such as air pollutants and allergens.[4–6] We therefore hypothesized that quantifying urinary eicosanoid levels in a controlled human exposure study to diesel exhaust (DE; a paradigm of traffic-related air pollution (TRAP)) and allergen would provide insight into pathophysiological responses known to contribute to asthma pathophysiology.

Environmental factors that contribute to asthma are those that induce airway hyperresponsiveness (AHR) and airway inflammation, both of which can be caused by allergens and exacerbated by air pollution exposures.[7–13] Inhaled exogenous eicosanoids such as prostaglandin D_2 (PGD_2) and cysteinyl leukotrienes (CysLTs) are potent bronchoconstrictors that are thought to be the main mediators of allergen-induced bronchoconstriction.[8,14–16] PGD_2 and leukotriene E_4 (LTE_4) are also potent chemoattractant for eosinophils and neutrophils, and mediate recruitment of these cells to airways.[17,18] Paracrine signaling of leukotrienes can lead to thromboxane synthesis in the airways following an allergen challenge.[19] Together, CysLTs, PGD_2, and thromboxanes are involved in regulating airway smooth muscle contraction, mucus secretion, plasma exudation, and altered mucosal blood flow of asthmatic airways.[19] These end organ effects mimic the pathophysiological features of asthma and are thought to be the driver of symptoms of asthma.

We previously demonstrated that coexposure to DE and allergen led to impaired airflow and increased AHR in allergen-sensitized and normally responsive individuals.[11] The coexposure also increased airway inflammation, which was highlighted by increased eosinophils, eotaxin-3, and interleukin-5 in bronchoalveolar lavage.[20] Therefore, we hypothesized that allergen and DE coexposure led to an increase in urinary levels of LTE_4 and PGD_2 metabolites, and that allergen-induced increases in the levels of those metabolites would correlate with airflow impairment and AHR.

We also sought to investigate factors that may modify the level of eicosanoids produced in response to allergen and DE exposures. To this end we evaluated whether having AHR or high genetic risk scores (GRS) would modify the exposure-

induced increase in urinary eicosanoids. In our previous report, allergen and DE coexposure increased airway responsiveness to methacholine only in the normally responsive individuals.[11] We reasoned that if the increased airway responsiveness is attributable to leukotrienes or PGD_2, then non-AHR participants would have a larger increase in LTE_4 or PGD_2 following the coexposure. In Wooding *et al*, we also demonstrated that GRS modified the effect of DE and allergen coexposure on circulating leukocytes, such that individuals with higher GRS had a greater increase in circulating leukocytes following the coexposure.[11] We reasoned that if the eicosanoids were a substantial contributor to exposure-induced inflammation, then GRS would also modify the exposure effects on urinary eicosanoids.

Environmental exposures are the predominant driver of the onset of respiratory disease.[21,22] There is accordingly an urgent need to understand the physiological responses to exposure, especially within the context of real-world mixed exposures. The current investigations subsequently comprised a focus inquiry into the effect of coexposures of allergen and DE upon the observed levels of urinary eicosanoids. Given the importance of these molecules in the pathophysiology of asthma and allergy, there is significant interest in further understanding the role of exposures to environmental mixtures upon their observed levels. For example, these effects could be important for attempts to utilize urinary eicosanoid profiles for patient stratification given the potential confounding effects upon quantified levels. These results highlight the importance of examining environmental exposures as mixtures *vs.* single exposures and further support the use of urinary eicosanoid concentrations to monitor changes in lung physiology.[23]

METHODS

Study Design

We performed a randomized, double-blinded, crossover, controlled exposure study to DE and allergen. The study was approved by the University of British Columbia Research Ethics Board (H11-01831), and all participants gave written informed consent. Fourteen allergen-sensitized participants aged 23-50 (7 male; 7 female) completed the study. All participants were self-reported never-smokers, and tested negative for urinary cotinine (<12 ng/mL). All participants showed a positive skin prick test for one or more aeroallergen used in the protocol (house dust mite (HDM), birch, or Timothy grass), and showed a 20% or greater decline in forced expiratory volume in 1 second (FEV_1) following the allergen inhalation challenge during the in-visit screening.

As shown in Figure 1, each participant was separately exposed to four coexposure conditions in random order, each separated by a 4-week washout period: 1) filtered air (FA) + saline (0.9% NaCl) (FA-S, negative control); 2) FA + allergen (FA-A); 3) DE diluted to 300 μg/m^3 of particulate matter with aerodynamic diameter <2.5 μm ($PM_{2.5}$) + allergen (DE-A); and 4) particle-depleted DE + allergen (PDDE-A). Exposure to FA, DE, and PDDE took place using the exposure system and protocol previously described.[11,20,24] PDDE was achieved by removing particulates from freshly generated DE using a combination of high-efficiency particulate air filtration and electrostatic precipitation.[11] Each participant was first exposed to FA, DE, or PDDE for 2 h and then went through the allergen inhalation challenge.[11,20] The allergen inhalation challenge was performed using standardized allergenic extracts for HDM (*Dermatophagoides pteronyssinus*), Timothy grass, or birch mix that were purchased from Hollister-Stier Laboratories (Spokane, WA, USA). Concentrated extract was diluted using saline immediately prior to each inhalation challenge. 2-minute inhaled allergen challenge was performed using an allergen provocation concentration to elicit 20% decrease in FEV_1 (PC_{20}) that was determined at screening based on methacholine PC_{20} and skin prick wheal size.[25]

GRS was calculated using variants (sixteen null alleles, micro insertion/deletion sites, and single nucleotide polymorphisms) that plausibly modulate the response to air pollution, as previously described.[11] The score is based on genes related to oxidative stress and related immune function responsive to TRAP.[26] Table 1 summarizes participant characteristics such as sex, FEV_1 % predicted, GRS, methacholine PC_{20}, and the allergen used in the study protocol.

Sample collection and storage

Urine samples were collected at baseline (before the exposure) and 4, 24, and 48 h after each exposure. Immediately after urine collection, samples were aliquoted, and butylated hydroxy toluene was added to achieve a final concentration of 227 μM. Samples were then stored at -80 °C until the day of analysis. We analysed 218 samples from 14 research participants. Six samples were missing due to insufficient volume of urine collected.

Urinary eicosanoid metabolites analysis

We assayed urinary eicosanoid metabolites using LC-MS/MS, using methodology described in detail in our previous publication.[27] Chromatographic separations were performed using a Waters Acquity UPLC system (Waters, Milford, MA). Data acquisition was performed using a triple quadrupole (Xevo TQ-S) mass spectrometer system (Waters). Peak detection, integration, and quantification were performed using the software Masslynx™ and TargetLynx™. The specific

gravity of the urine was used to normalize quantified eicosanoid metabolite levels relative to urinary excretion volume as previously described.[27]

Statistical analyses

Effects of exposures on urinary eicosanoid metabolites were estimated using linear mixed-effects (LME) models (nlme package; version 3.1-147) in R (version 4.0.3) using R Studio (version 1.3.1093). Confidence intervals (CI) were computed using the gmodels package (version 2.18.1) in R. In our primary LME model, condition (FA-S, FA-A, DE-A, or PDDE-A) was used as fixed effect and participant ID was set as random effect.

To test the hypothesis that individuals *without AHR* (defined by methacholine PC_{20} greater than 8 mg/mL), would accumulate more eicosanoid metabolites in response to the allergen and DE exposures, a second model was used where condition-by-AHR interaction was the fixed effect. To test our hypothesis that individuals with higher GRS would accumulate more eicosanoid metabolites in response to the allergen and DE exposures, a third model was used where condition-by-GRS interaction was the fixed effect.

When necessary to normalize the data distribution, data were $\log_{10}$-transformed. Effect estimates and associated confidence intervals are presented as back-log transformed values. Therefore, a given effect estimate represents the ratio of fold changes induced by a given condition relative to control (FA-S), unless noted otherwise.

To assess the correlation between eicosanoid metabolites with PC_{20} and FEV_1, we performed repeated measures correlation analysis using the rmcorr package (version.0.3.1) in R. Each repeated measures correlation coefficient (r) is reported with the 95% CI and p-value.

P-values less than 0.05 were considered statistically significant. Results are presented unadjusted for multiple comparisons, given that our primary intention is not to interpret statistical significance as a threshold determinant of critical importance but rather to observe patterns within pathways (whose family members are not independent) so as to enhance our understanding of the coexposure effects we have previously observed. In addition, the LC-MS/MS method employed covers a total of 7 synthetic pathways to produce eicosanoids,[27] resulting in a potential for 0.35 false positives at alpha=0.05.

RESULTS

Sixteen eicosanoids were detected in the urine of allergen-sensitized individuals

Sixteen different lipid mediators including metabolites of prostaglandins (PGE_2, tetranor PGEM, 6-keto-$PGF_{1\alpha}$, 2,3-dinor-6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$, and 13,14-dihydro-15-keto$PGF_{2\alpha}$, 2,3-dinor-11-β-$PGF_{2\alpha}$, tetranor PGDM), thromboxanes (TXB_2, 2,3-dinor-TXB_2, and 11-dehydro-TXB_2), cysteinyl leukotrienes (LTE_4), and isoprostanes (8-iso$PGF_{2\alpha}$, 8,12-iso-i$PF_{2\alpha}$-VI, 5-i$PF_{2\alpha}$-VI and 2,3-dinor-8-iso $PGF_{2\alpha}$) were detected in urine.

Allergen inhalation increased LTE_4 in urine

Table 2 summarizes the effect of three exposures on the CysLT urinary metabolite LTE_4. There was a significant increase in LTE_4 in urine 4 h after FA-A, DE-A, and PDDE-A exposures compared to FA-S (Figure 2A), and this persisted to 48 h in FA-A alone.

Allergen inhalation and DE exposure increased prostacyclin pathway metabolites, and PDDE blocked conversion of 6-keto-$PGF_{1\alpha}$ into 2,3-dinor-6-keto-$PGF_{1\alpha}$

At 24 h post-exposure, both FA-A and DE-A exposure led to significant accumulation of 2,3-dinor-6-keto-$PGF_{1\alpha}$ compared to FA-S (Figure 2F). At the same timepoint, there was a significant increase in 6-keto-$PGF_{1\alpha}$ after PDDE-A exposure compared to FA-S (1.33 [1.00 to 1.75]), while there was no detectable increase of 2,3-dinor-6-keto-$PGF_{1\alpha}$ elicited by PDDE-A exposure (Figure 2F). Indeed, there was significantly lower 2,3-dinor-6-keto-$PGF_{1\alpha}$ in urine after PDDE-A compared to DE-A (0.3 [0.13 to 0.71]).

Allergen inhalation increased urinary metabolites of the PGD_2 and TXA_2 pathways, and AHR and GRS modified the allergen effect on those metabolites

Allergen inhalation significantly increased a PGD_2 metabolite (2,3-dinor-11β-$PGF_{2\alpha}$ at 4 h and tetranor PGDM at 4 and 24 h), with no detectable additional impact by the exposure to DE or PDDE (Figure 2B&C; Table 2). At 4 h post-exposure, there were significant condition-by-AHR interactions such that participants without AHR experienced a greater increase in 2,3-dinor-11β-$PGF_{2\alpha}$ after the FA-A or DE-A exposures compared to those without AHR. This pattern was also consistent at 24 h post-exposure; however, it was driven by a different PGD_2 metabolite (tetranor PGDM instead of 2,3-dinor-11β-$PGF_{2\alpha}$); participants with AHR experienced a greater increase in tetranor PGDM after the FA-A or DE-A

exposures compared to those without AHR. There was significant condition-by-GRS interaction at 4 h such that participants with higher GRS experienced greater increase levels of the PGD_2 metabolite 2,3-dinor-11β-$PGF_{2\alpha}$ after inhaling allergen-alone.

At 24 h post-exposure, allergen inhalation increased two thromboxane metabolites (2,3-dinor-TXB_2 and 11-dehydro-TXB_2; Table 2; Figure 2D). There was significant condition-by-AHR interaction such that participants without AHR experienced greater increase in 11-dehydro-TXB_2 elicited by FA-A exposure at 4 and 24 h post-exposure. There was significant condition-by-GRS interaction such that participants with higher GRS score experienced higher increase in thromboxane metabolites (2,3-dinor-TXB_2 and 11-dehydro-TXB_2) at 24 h post-exposure. There was a significant increase in 11-dehydro-TXB_2 at 24 h after DE-A (1.96 [1.37 to 2.80]) and PDDE-A (1.61 [1.10 to 2.35]) compared to FA-S (Table 2 & Figure 2D).

Allergen inhalation increased isoprostanes in urine, and GRS modified the effect of DE and allergen coexposure

At 24 h post allergen inhalation, we saw a significant increase of 5-i$PF_{2\alpha}$-VI (1.32 [1.10 to 1.58]), 8-*iso*-$PGF_{2\alpha}$ (1.44 [1.03 to 2.02]) and 8,12-*iso*-i$PF_{2\alpha}$-VI (1.24, [1.05 to 1.48]) in FA-A compared to FA-S. There was no detectable additional impact of DE-A and PDDE-A exposures on any of the four isoprostanes compared to the allergen alone exposure. There was significant condition-by-GRS interaction for DE-A versus FA-S, and DE-A versus FA-A comparisons such that participants with higher GRS had greater increase of 8-*iso*-$PGF_{2\alpha}$ and 2,3-dinor-8-*iso*-$PGF_{2\alpha}$ than those with lower GRS at 24 h.

Levels of tetranor PGDM and LTE_4 in urine correlated with airway responsiveness and change in FEV_1

At 24 h post-exposure, urinary tetranor PGDM correlated with log PC_{20} (r = -0.49 [-0.705 to -0.192]; p=0.002) and the change in FEV_1 % predicted from baseline to 24 h (r = -0.402 [-0.636 to -0.098]; p=0.01). At the same timepoint, LTE_4 (r = -0.424 [-0.660 to -0.111]; p=0.008) and 2,3-dinor-8-*iso*-$PGF_{2\alpha}$ (r = 0.385 [0.064 to 0.633]; p=0.02) correlated with log PC_{20}. The isoprostane 5-i$PF_{2\alpha}$-VI (r = -0.334 [-0.588 to -0.021]; p=0.03) and 2,3-dinor-TXB_2 (r = -0.324 [-0.58 to -0.009]; p=0.03) correlated with the FEV_1 change. There was no significant correlation between the concentrations of other urinary eicosanoid metabolites and log PC_{20} or the change in FEV_1 % predicted.

DISCUSSION

In this controlled human exposure study, we monitored and detailed the changes in urinary eicosanoids over 48 h following exposure to FA-S (negative control), FA-A (allergen alone), DE-A (full coexposure), and PDDE-A (coexposure with minimal particles). We demonstrated some expected allergen-induced changes, provided insight not previously delineated using a controlled human model, and further documented novel findings regarding modifying effects of coexposure to pollution, reduction of pollution particles, airway responsiveness, and genetic predisposition. Collectively, these findings deepen our understanding of airway responses to common exposures with appreciation for several important factors not usually incorporated into such analyses.

In line with our previous finding from the segmental allergen challenge model,[27] we observed that allergen inhalation led to an increase of LTE_4 in urine at 4 h post-exposure. In asthmatics, leukotrienes can induce airflow obstruction by causing bronchoconstriction, mucosal edema, and mucus hypersecretion.[14,28] LTE_4 can also induce mast cell activation, which leads to the release of the mast cell mediator PGD_2.[14] Paracrine signaling of leukotrienes can lead to thromboxane synthesis in the airways following an allergen challenge, which occurs as the secondary effect to $CysLT_1$ receptor activation on cells within the airway wall.[19] As expected, we observed that increases in LTE_4 were accompanied by a concomitant increase in the PGD_2 urinary metabolite tetranor PGDM and increases in thromboxane metabolites (2,3-dinor-TXB_2, 11-dehydro-TXB_2) and $PGF_{2\alpha}$ after inhalation of allergen.

We found that participants without AHR, as defined by having methacholine PC_{20} >8 mg/mL, experienced greater increases in tetranor PGDM after FA-A and DE-A exposures compared to those with AHR. We also found that there was significant condition-by-AHR interaction ($p<0.05$) for FA-A *vs.* FA-S comparison such that participants without AHR experienced greater decreases in FEV_1 % 24 h following allergen inhalation (data not shown). Given that PGD_2 is downstream of $CysLT_1$ receptor activation by leukotrienes in mast cells,[14] and urinary tetranor PGDM reflects the biosynthesis of PGD_2,[29] our data suggests that individuals that were normally responsive to methacholine were more sensitive to allergen-induced increase in LTE_4, resulting in a greater accumulation of tetranor PGDM, and greater decrease in FEV_1 % compared to normally responsive individuals. Similar to tetranor PGDM, increases of 11-dehydro-TXB_2 were also smaller in those with AHR compared to those without AHR.

Our observations are in agreement with previous studies demonstrating that individuals with highest hyperresponsiveness to methacholine had the lowest relative airway responsiveness to LTE_4, LTC_4 or LTD_4.[14,16,30] In our study, urinary LTE_4 and tetranor PGDM were negatively correlated with PC_{20}, which reflects the airway responsiveness. An increased level of urinary tetranor PGDM negatively correlated with the change in FEV_1 following allergen inhalation, such that higher urinary tetranor PGDM correlated with greater allergen-induced airflow limitation. Knowing that inhaled PGD_2 can in addition to directly causing bronchoconstriction also potentiate AHR to methacholine and histamine, [15] we can speculate that acquired AHR observed in normally responsive individuals after the exposure to DE-A and PDDE-A is partly driven by the PGD_2 secreted in the airways following the coexposure. We reported that the decrease in PC_{20} was observed only in the normally responsive individuals after the exposure to DE-A and PDDE-A.[11] Taken together, our data supports that the allergen-sensitized individuals without underlying AHR may experience a greater compromise of airflow and increase in airway sensitivity to contractile triggers following exposure to allergens and this effect may be mediated through leukotrienes and prostaglandins. This may explain previous studies showing such coexposures being a strong risk factor for the development of *de novo* asthma.[31]

Another main aim of our study was to evaluate a plausible gene-environmental interaction in modulating the exposure-effects upon eicosanoids. We hypothesized that a rationally focused GRS would modify the effect of allergen inhalation and DE exposure on eicosanoids. The chosen allelic variants used in calculating GRS were suggested by epidemiological studies to modulate the response to air pollution.[32–34] The GRS was calculated as the unweighted sum of the number of SNP risk alleles and null for GSTT1 or GSTM1.[11] We previously reported that GRS modified the effect of allergen and DE coexposures on peripheral monocyte counts such that individuals with higher GRS score was associated with a greater increase in the cell counts after coexposures.[11] Indeed, we found significant condition-by-GRS interaction for thromboxane metabolites (2,3-dinor-TXB_2, 11-dehydro-TXB_2) and a PGD_2 metabolite (2,3-dinor-11β-$PGF_{2\alpha}$) such that a higher GRS was associated with a greater increase in these metabolites after the allergen-alone exposure. We also found that there was significant condition-by-GRS interaction for 2 isoprostanes (2,3-dinor-8-*iso*-$PGF_{2\alpha}$, 8-*iso*-$PGF_{2\alpha}$) such that a higher GRS was associated with a greater increase after DE-A. Given that both thromboxane and PGD_2 are involved in mediating acute physiological changes that highlight the pathophysiological features of asthma, our data provides further experimental evidence that air pollution susceptibility genes discovered through large epidemiological studies may modulate the pathophysiological responses to the environmental exposures in allergen-sensitized individuals. Therefore,

our findings highlight the importance of studying the gene-environment interaction contributing to asthma pathophysiology. However, these results should be interpreted with a caution due to the small sample size of our study.

Perhaps one of the most striking findings of our study was that DE-A coexposure increased prostacyclin (PGI_2) pathway metabolites, and PDDE ablated conversion of 6-keto-$PGF_{1\alpha}$ into 2,3-dinor-6-keto-$PGF_{1\alpha}$. The eicosanoid prostacyclin is derived from arachidonic acid via cyclooxygenase (COX)-2 and PGI synthase (PGIS) activity.[35] Prostacyclin regulates the innate and adaptive immune systems, and its role is thought to be primarily anti-inflammatory in nature.[36] Inability to signal through the PGI_2 cell surface receptor has been shown to enhance acute and chronic allergic inflammation and airway remodeling in a mouse model.[37,38] DE inhalation has been shown to cause an imbalance between PGI_2 and TXA_2 production, favouring more COX-2 mediated PGI_2 production in mice.[39]

In humans, inhalation of PGI_2 has been shown in asthmatics to protect against bronchoconstriction provoked by PGD_2 or methacholine.[40] Therefore, alteration of the PGI_2 pathway by TRAP may explain our observation of acquired AHR in normally responsive individuals following the exposure to DE-A and PDDE-A. Indeed, we found that PDDE-A impaired FEV_1 to a larger extent than DE-A in the first 30 minutes after the allergen challenge.[11] We also reported that there was evidence of increased epithelial-blood barrier damage in the lungs by PDDE-A exposure compared to DE-A, and this may be attributable to the suppressed PGI_2.[20] However, we do not have conclusive evidence in this study to confirm that imbalance between PGI_2 and TXA_2 production, or the suppression of PGI_2, led to the acquired AHR or loss of epithelial-blood barrier function. Pharmacological studies inhibiting these pathways in an animal model would be more appropriate to provide deeper mechanistic understanding. Nonetheless, our observations highlight that air pollution reduction technology should focus on reducing both the particulates and gases of the diesel emissions.

The strength of our study lies in utilizing the robust quantitative metabolic profiling of urinary eicosanoids in a randomized, double-blinded, crossover study. Since individuals served as their own baseline values, we were able to focus on the intra-individual's changes in observed eicosanoid levels following exposures, enabling the detection of statistically significant changes with only fourteen research participants. Moreover, the crossover design allowed for testing of effect modifications by AHR and GRS. In the context of TRAP exposures, gene-environment interaction appears to modulate eicosanoid-mediated physiological responses known to contribute to asthma pathophysiology. However, because we performed multiple statistical comparison of eicosanoids that are highly correlated with each other, there is multiplicity issues that may have potentially inflated the type 1 error rate as a result of multiple testing. It should be highlighted

though, that all eicosanoids are produced via a single enzymatic pathway involving the release of arachidonic acid from the membrane via phospholipase A_2 activity. Accordingly, given that eicosanoids are not independent variables, results are presented unadjusted for multiple comparisons. Future clinical studies utilizing eicosanoids as a biomarker should clearly determine *a priori* eicosanoid metabolite(s) hypothesized to be modulated by environmental exposures and/or intervention. In this regard, this study adds to the literature by identifying specific eicosanoid metabolites that may be chosen in a future study to objectively measure the impact of more complex exposures and interventions. Our findings further support the use of urinary eicosanoid levels to monitor the effects of lung pathophysiology and inhalation studies, suggesting their utility for environmental exposures studies.

Author contributions

MHR managed the clinical study, acquired data, and performed statistical analysis and interpretation of results. MHR drafted and edited the manuscript. CG performed mass spectrometry and edited the manuscript. ACYY recruited research participants, managed the study, collected clinical data, and edited the manuscript. CW contributed to conception and design, analysis and interpretation of data. CC supervised the clinical study. All authors have given final approval of the manuscript and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

TABLES

Table 1. Participant genetic risk score, methacholine PC20 and allergen used

	Sex	FEV_1 % Predicted	Genetic Risk Score	Methacholine PC_{20} (mg/mL)	Allergen (Concentration)
1	M	108	15	>128	HDM (1/32)
2	F	105	12	>128	HDM (1/32)
3	F	111	5	5.9	HDM (1/128)
4	M	104	7	2.6	HDM (1/512)
5	F	100	7	0.9	HDM (1/16384)
6	M	107	12	6.8	HDM (1/512)
7	F	114	6	6.9	HDM (1/4)
8	F	97	13	>128	Grass (1/256)
9	M	105	9	>128	Grass (1/1024)
10	M	102	6	0.5	Grass (1/66538)
11	M	86	9	0.8	Grass (1/4096)
12	F	84	12	1.6	Grass (1/256)
13	F	122	N/A	>128	Birch (1/64)
14	M	123	10	3.5	Birch (1/8)
		Mean ± SD			
	7 M 7 F	105 ± 11	8 ± 3	5 normally responsive 9 hyper-responsive	7 HDM 5 Grass 2 Birch

Abbreviations: HDM = house dust mite; FEV_1 = forced expiratory volume in 1 second; PC_{20} = provocative concentration causing 20% decrease in FEV_1.

Table 2. Effect of exposures on urinary eicosanoid metabolites

		FA-A		DE-A		PDDE-A	
	Time (h)	**Effect [95% CI]**	**p value**	**Effect [95% CI]**	**p value**	**Effect [95% CI]**	**p value**
LTE pathway							
LTE_4	4	2.91 [1.72 to 4.93]	**0.0002**	2.41 [1.41 to 4.14]	**0.002**	2.42 [1.41 to 4.14]	**0.002**
	24	1.48 [1.06 to 2.07]	**0.02**	1.19 [0.86 to 1.66]	0.29	1.22 [0.86 to 1.73]	0.25
	48	1.49 [1.02 to 2.20]	**0.04**	1.30 [0.89 to 1.91]	0.17	1.25 [0.83 to 1.87]	0.27
PGE_2 pathway							
PGE_2	4	1.04 [0.69 to 1.58]	0.83	1.34 [0.87 to 2.05]	0.18	1.30 [0.85 to 1.99]	0.22
	24	1.25 [0.65 to 2.40]	0.50	0.64 [0.33 to 1.23]	0.18	0.77 [0.39 to 1.52]	0.44
	48	0.48 [0.20 to 1.14]	0.10	0.77 [0.33 to 1.81]	0.54	0.45 [0.18 to 1.11]	0.08
Tetranor PGEM	4	0.91 [0.62 to 1.33]	0.62	0.95 [0.64 to 1.40]	0.78	1.11 [0.76 to 1.64]	0.58
	24	1.45 [0.97 to 2.18]	0.07	1.23 [0.82 to 1.84]	0.32	1.10 [0.72 to 1.69]	0.66
	48	1.08 [0.64 to 1.84]	0.76	0.72 [0.42 to 1.22]	0.22	0.91 [0.52 to 1.58]	0.73
PGI_2 pathway							
6-keto-$PGF_{1\alpha}$	4	1.19 [0.82 to 1.75]	0.35	0.95 [0.65 to 1.41]	0.81	1.00 [0.68 to 1.47]	0.99
	24	1.05 [0.81 to 1.38]	0.69	1.23 [0.94 to 1.61]	0.12	1.33 [1.00 to 1.75]	**0.047**
	48	0.74 [0.51 to 1.07]	0.11	0.88 [0.61 to 1.28]	0.51	1.11 [0.75 to 1.63]	0.59
2,3-dinor-6-keto-$PGF_{1\alpha}$	4	1.46 [0.49 to 4.34]	0.48	1.65 [0.54 to 4.99]	0.37	3.08 [1.02 to 9.34]	**0.047**
	24	4.00 [1.79 to 8.95]	**0.001**	6.98 [3.12 to 15.6]	**<0.0001**	2.12 [0.91 to 4.92]	0.08
	48	1.15 [0.43 to 3.07]	0.78	1.61 [0.60 to 4.30]	0.33	1.38 [0.49 to 3.88]	0.53
$PGF_{2\alpha}$ pathway							
$PGF_{2\alpha}$	4	1.05 [0.80 to 1.38]	0.72	1.04 [0.78 to 1.37]	0.79	1.18 [0.89 to 1.56]	0.25
	24	1.32 [1.01 to 1.71]	**0.04**	1.36 [1.05 to 1.77]	**0.02**	1.22 [0.92 to 1.61]	0.16
	48	0.73 [0.43 to 1.24]	0.24	0.54 [0.32 to 0.91]	**0.02**	0.75 [0.43 to 1.30]	0.29
13,14-dihydro-15-keto-$PGF_{2\alpha}$	4	1.11 [0.91 to 1.36]	0.30	1.04 [0.85 to 1.28]	0.68	1.02 [0.83 to 1.26]	0.82
	24	1.10 [0.85 to 1.41]	0.47	1.17 [0.91 to 1.51]	0.21	1.11 [0.85 to 1.45]	0.43
	48	0.84 [0.51 to 1.41]	0.51	0.67 [0.40 to 1.12]	0.12	0.92 [0.54 to 1.57]	0.75
PGD_2 pathway							
2,3-dinor-11β-$PGF_{2\alpha}$	4	2.22 [1.23 to 4.01]	**0.01**	1.72 [0.94 to 3.16]	0.08	1.89 [1.03 to 3.48]	**0.04**
	24	1.37 [0.75 to 2.49]	0.30	1.25 [0.69 to 2.28]	0.45	1.43 [0.76 to 2.68]	0.26
	48	1.10 [0.61 to 1.99]	0.73	1.57 [0.87 to 2.84]	0.13	1.17 [0.63 to 2.16]	0.62
Tetranor PGDM	4	1.90 [1.22 to 2.95]	**0.006**	1.89 [1.20 to 2.96]	**0.007**	2.12 [1.35 to 3.32]	**0.002**
	24	1.66 [1.32 to 2.09]	**0.0001**	1.55 [1.24 to 1.96]	**0.0004**	1.50 [1.18 to 1.90]	**0.002**
	48	1.23 [0.95 to 1.60]	0.11	1.14 [0.88 to 1.48]	0.31	1.24 [0.94 to 1.63]	0.12
TXA_2 pathway							
TXB_2	4	1.35 [0.79 to 2.28]	0.26	1.36 [0.79 to 2.34]	0.25	1.55 [0.90 to 2.66]	0.11
	24	1.24 [0.65 to 2.40]	0.50	1.03 [0.53 to 1.98]	0.93	1.22 [0.61 to 2.42]	0.57
	48	0.91 [0.40 to 2.10]	0.83	1.06 [0.46 to 2.44]	0.89	0.82 [0.34 to 1.95]	0.64
	4	1.98 [1.14 to 3.43]	**0.02**	1.69 [0.96 to 2.98]	0.07	1.58 [0.90 to 2.78]	0.11

2,3-dinor-TXB_2	24	1.83 [1.22 to 2.74]	**0.005**	1.47 [0.98 to 2.20]	0.06	1.45 [0.95 to 2.23]	0.08
	48	1.10 [0.73 to 1.64]	0.64	0.95 [0.64 to 1.42]	0.80	0.97 [0.64 to 1.49]	0.90
11-dehydro-TXB_2	4	1.76 [1.06 to 2.92]	**0.03**	1.55 [0.92 to 2.60]	0.09	1.20 [0.71 to 2.01]	0.49
	24	2.39 [1.67 to 3.42]	**<0.0001**	1.96 [1.37 to 2.80]	**0.0005**	1.61 [1.10 to 2.35]	**0.01**
	48	0.88 [0.59 to 1.30]	0.50	0.85 [0.57 to 1.26]	0.41	0.93 [0.61 to 1.41]	0.72
Isoprostanes							
8-*iso*-$PGF_{2\alpha}$	4	1.22 [0.90 to 1.65]	0.19	1.18 [0.86 to 1.60]	0.30	1.17 [0.86 to 1.60]	0.31
	24	1.44 [1.03 to 2.02]	**0.03**	1.42 [1.02 to 2.00]	**0.04**	1.25 [0.88 to 1.78]	0.21
	48	0.85 [0.49 to 1.48]	0.56	1.14 [0.66 to 1.97]	0.63	1.00 [0.56 to 1.77]	0.99
2,3-dinor-8-*iso*-$PGF_{2\alpha}$	4	0.98 [0.74 to 1.31]	0.89	0.79 [0.59 to 1.06]	0.11	0.90 [0.67 to 1.21]	0.50
	24	0.96 [0.70 to 1.31]	0.77	0.84 [0.62 to 1.15]	0.28	0.82 [0.59 to 1.14]	0.23
	48	1.20 [0.79 to 1.82]	0.39	0.96 [0.63 to 1.46]	0.85	1.00 [0.64 to 1.55]	0.99
5-$iPF_{2\alpha}$-VI	4	1.24 [0.98 to 1.58]	0.07	1.09 [0.85 to 1.39]	0.50	1.03 [0.81 to 1.32]	0.78
	24	1.32 [1.10 to 1.58]	**0.004**	1.11 [0.93 to 1.33]	0.25	1.23 [1.02 to 1.49]	**0.03**
	48	0.87 [0.70 to 1.07]	0.17	0.98 [0.79 to 1.20]	0.82	0.91 [0.73 to 1.13]	0.39
8,12-iso-$iPF_{2\alpha}$ -VI	4	1.12 [0.90 to 1.39]	0.29	1.11 [0.89 to 1.39]	0.33	1.16 [0.93 to 1.44]	0.18
	24	1.24 [1.04 to 1.49]	**0.02**	1.15 [0.96 to 1.37]	0.13	1.18 [0.98 to 1.42]	0.08
	48	0.81 [0.66 to 0.99]	**0.04**	0.90 [0.73 to 1.10]	0.29	0.91 [0.73 to 1.13]	0.37

Abbreviations: CI = confidence interval; three exposure conditions were: FA-A = filtered air allergen; DE-A = diesel exhaust and allergen; PDDE-A = particle depleted diesel exhaust and allergen; LTE_4 = leukotriene E_4; PGE_2 = prostaglandin E_2; tetranor PGEM = tetranor prostaglandin E_2 metabolite; PGI_2 = prostacyclin; $PGF_{1\alpha}$ = prostaglandin F_{1alpha}; $PGF_{2\alpha}$ = prostaglandin F_{2alpha}; PGD_2 = prostaglandin D_2; tetranor PGDM = tetranor prostaglandin D_2 metabolite; TXA_2 = thromboxane A_2; TXB_2 = thromboxane B_2; $PGF_{2\alpha}$ = prostaglandin F_2alpha; $iPF_{2\alpha}$ = isoprostane F_2alpha.

Effect of exposures (and confidence intervals) are differences in fold changes relative to those observed in the control (FA-S). Significant exposure effects ($p<0.05$) are bolded.

FIGURES

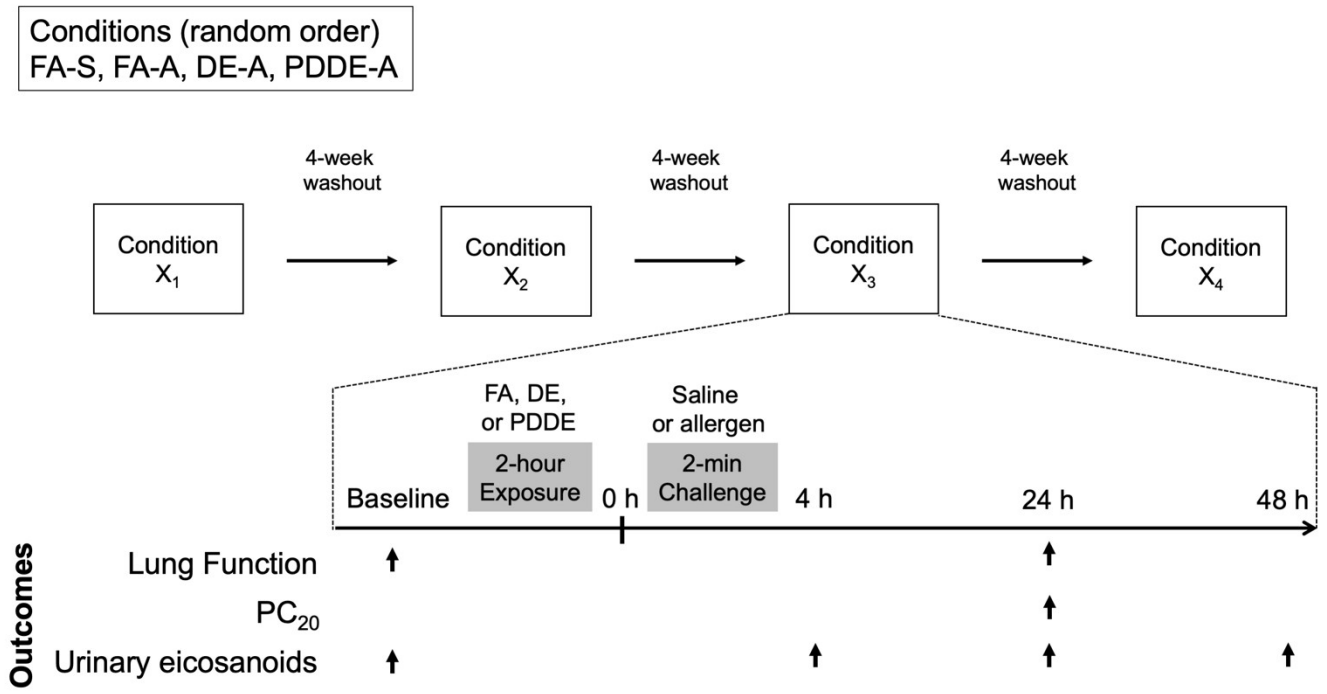

Figure 1. Schematic summary of experimental design and outcomes. Participants were confirmed to be sensitized to one or more allergens (house dust mite, grass, and birch) during screening. In this double-blinded, randomized, crossover study, each research participant was exposed in random order, each separated by a 4-week washout period to four conditions: FA-S = filtered air saline; FA-A = filtered air allergen; DE-A = diesel exhaust and allergen; PDDE-A = particle depleted diesel exhaust and allergen. Outcomes measured were forced expiratory volume in 1 second (FEV_1) during lung function testing, methacholine provocative concentration eliciting 20% decrease in FEV_1 (PC_{20}), and urinary eicosanoid metabolites measured using LC-MS/MS.

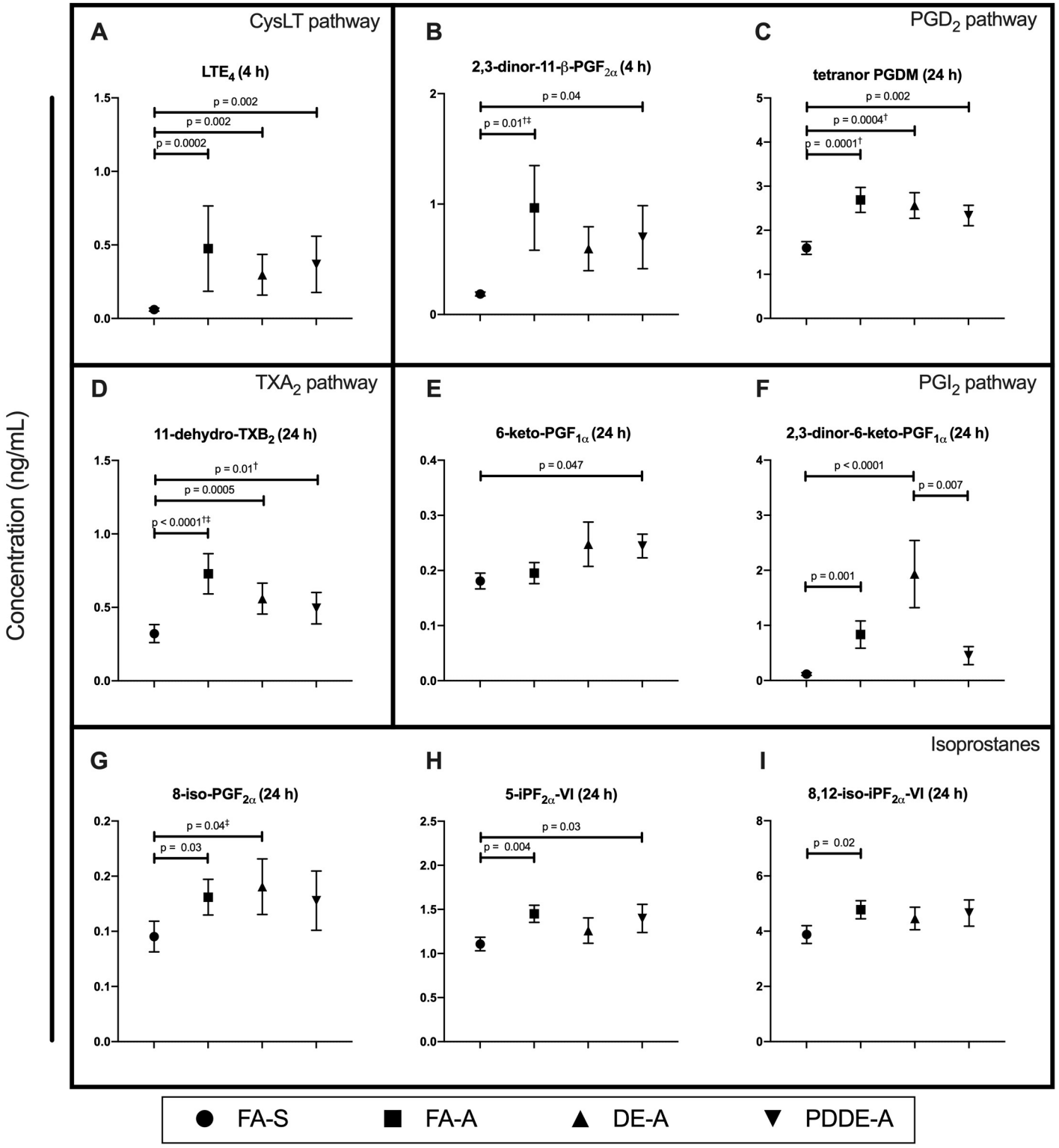

Figure 2. Effects of exposure to diesel exhaust and allergen on urinary eicosanoids. The four experimental conditions were: FA-S (filtered air + saline); FA-A (filtered air + allergen); DE-A (diesel exhaust + allergen); PDDE-A (particle-depleted diesel exhaust + allergen). Data are presented as mean and SEM (4 h data, n = 14 for FA-S, FA-A, and PDDE-A, n=13 for DE-A; and, 24 h data, n = 14 for FA-S and FA-A, n = 13 for DE-A, n = 12 for PDDE-A). Graphs are organized

into panels: **(A)** cysteinyl leukotriene (CysLT) pathway showing leukotriene E_4 (LTE_4); **(B&C)** prostaglandin E_2 (PGE_2) pathway showing 2,3-dinor-11-β-prostaglandin F2alpha (-$PGF_{2\alpha}$) and tetranor prostaglandin D_2 metabolite tetranor (PGDM); **(D)** thromboxane A2 (TXA_2) pathway showing 11-dehydro-thromboxane B2 (-TXB_2); **(E&F)** prostacyclin (PGI_2) pathway showing 6-keto-prostaglandin F1alpha (-$PGF_{1\alpha}$) and 2,3-dinor-6-keto-$PGF_{1\alpha}$; and **(G,H,I)** isprostanes showing 8-iso-$PGF_{2\alpha}$, 5-isoprostane F2alpha (-$iPF_{2\alpha}$)-VI, and 8,12-$iPF_{2\alpha}$-VI. Statistical comparisons were done using linear mixed-effects models. † denotes significance additionally for condition-by-airway hyperresponsivness (AHR) interaction for that comparison, such that participants without AHR experienced greater increase of eicosanoid being compared. ‡ denotes significance additionally for condition-by-gene risk score (GRS) interaction for that comparison, such that participants with higher GRS experienced greater increase of eicosanoid being compared.

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