

UC Davis

UC Davis Previously Published Works

Title

Inhalation of Distinct Emission Components Comprising Traffic-Related Air Pollution Elicits Sex-Specific Effects on Pulmonary and Systemic Inflammation in the TgF344-AD Transgenic Rat Model of Alzheimer's Disease

Permalink

<https://escholarship.org/uc/item/32r0w10b>

Journal

Environment & Health

ISSN

2833-8278

Authors

Park, Heui Hye
Cheng, Mei-Yun
Valenzuela, Anthony E
[et al.](#)

Publication Date

2026-09-22

DOI

10.1021/envhealth.6c00178

Copyright Information

This work is made available under the terms of a Creative Commons Attribution-NonCommercial-NoDerivatives License, available at <https://creativecommons.org/licenses/by-nc-nd/4.0/>

Peer reviewed

Inhalation of Distinct Emission Components Comprising Traffic-Related Air Pollution Elicits Sex-Specific Effects on Pulmonary and Systemic Inflammation in the TgF344-AD Transgenic Rat Model of Alzheimer's Disease

Heui Hye Park, Mei-Yun Cheng, Anthony E. Valenzuela, Morgan C. Domanico, Christopher Wallis, Allyson Ikeda, Lourdes Sanchez, Allison Ehrlich, Rachel Reader, Vidya Chandrasekaran, Anthony S. Wexler, Keith J. Bein, Pamela J. Lein, and Laura S. Van Winkle*



Cite This: <https://doi.org/10.1021/envhealth.6c00178>



Read Online

ACCESS |



Metrics & More



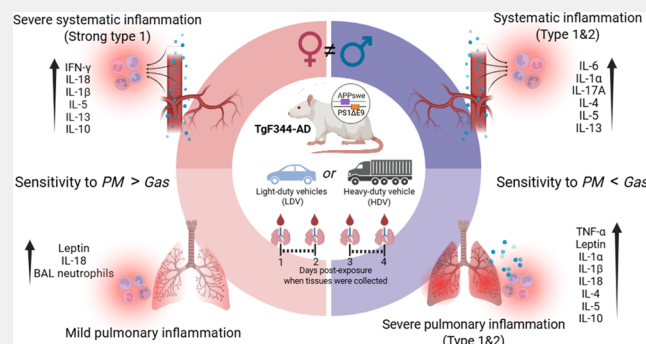
Article Recommendations



Supporting Information

ABSTRACT: Air pollution is associated with not only respiratory but also neurodegenerative diseases, such as Alzheimer's disease (AD). While underlying mechanisms remain unclear, the lung–brain axis has been proposed as a potential pathway in which pulmonary inflammation triggered by air pollution promotes systemic inflammation, which in turn triggers neuroinflammation to promote AD pathogenesis. This study defines chronic effects of ambient traffic-related air pollution (TRAP) and the contribution of TRAP-derived particulate matter (PM) vs gases, on peripheral inflammation in a transgenic rat model of AD. Female and male TgF344-AD rats that overexpress human AD risk genes were exposed for 3 months to TRAP drawn directly from a major freeway tunnel in Northern California, USA and delivered in real-time to animals housed in an exposure facility adjacent to the tunnel. Animals were exposed to filtered air, unfractionated TRAP (PM + Gas), or TRAP fractionated into PM-only or gas-only in the emissions of either a combination of light- and heavy-duty vehicles (LDV + HDV) or LDV-only. Our assessment of inflammatory mediators in the plasma and lung revealed sex- and component-specific TRAP effects. Overall, TgF344-AD females developed type 1 systemic inflammation in response to PM(LDV + HDV), while TgF344-AD males developed pulmonary type 1 inflammation and exhibited stronger type 2 adaptations, largely driven by exposure to Gas(LDV + HDV). Transgenic males had substantial increases in circulating HMGB1, but unchanged pulmonary HMGB1, across all TRAP exposures, whereas transgenic females had no changes in circulating HMGB1 but elevated pulmonary HMGB1 in the PM + Gas(LDV + HDV) group. While we expected synergistic or additive effects of PM and gas components on biological outcomes, we observed distinct inflammatory profiles for each component. Exposure to PM or gas fractions of TRAP induced more robust biological responses than the combined exposure to PM + Gas. These findings highlight the importance of studying the interactions of emission source-, pollutant type-effects of TRAP by sex on inflammation to understand multi-organ responses.

KEYWORDS: traffic-related air pollution, PM, gas vapor, pulmonary inflammation, systemic inflammation, cytokines, chronic inhalation exposure



INTRODUCTION

Epidemiologic and experimental studies have linked air pollution to negative health outcomes in the respiratory and cardiovascular systems.^{1,2} More recently, epidemiologic evidence has reported associations between air pollution and other organ systems, including the brain, kidney, and gut microbiome.^{3–5} In particular, increased risk of Alzheimer's disease (AD) and related dementias has been strongly associated with higher levels of air pollution in multiple epidemiologic studies across large and diverse cohorts.^{3,6,7}

Many experimental animal studies support these associations, demonstrating that TRAP exacerbated Alzheimer's phenotypes, such as amyloid deposition, blood–brain barrier (BBB)

Received: March 19, 2026

Revised: August 30, 2026

Accepted: August 31, 2026



ACS Publications

© XXXX The Authors. Co-published by
Research Center for Eco-Environmental
Sciences, Chinese Academy of Sciences,
and American Chemical Society

A

<https://doi.org/10.1021/envhealth.6c00178>
Environ. Health XXXX, XXX, XXX–XXX

Table 1. Exposure Groups^a

	exposure group	TRAP component	air source	sex	exposure <i>n</i>	analysis <i>n</i>
1	FA	N/A	air around the facility	F, M	9 per sex	4–9 per sex
2	PM + Gas(LDV + HDV)	PM + Gas	bore 1 (LDV + HDV)	F, M	9 per sex	4–9 per sex
3	Gas(LDV + HDV)	gas	bore 1 (LDV + HDV)	F, M	9 per sex	4–9 per sex
4	PM(LDV + HDV)	PM	bore 1 (LDV + HDV)	F, M	9 per sex	4–9 per sex
5	PM + Gas(LDV)	PM + Gas	bore 2 (LDV-only)	F, M	9 per sex	4–9 per sex
6	PM(LDV)	PM	bore 2 (LDV-only)	F, M	9 per sex	4–9 per sex

^aAnalysis *n* after exclusion of statistical outliers, the sample size used for analyses ranged from *n* = 4–9 per group per sex, depending on the outcome.

impairment, and glial activation in rodents following acute and chronic exposures to resuspended, laboratory generated, or real-world PM extracts or air pollutants.^{3,8–11} However, the question of whether chronic human-relevant exposures to air pollution promote AD-relevant neurological outcomes and the mechanisms underlying these associations have not yet been elucidated. The lung–brain axis hypothesis¹² has prompted animal studies to investigate mediating factors connecting air pollution effects on the lung to adverse brain outcomes. However, the complex inflammatory crosstalk between the lung, circulatory system, and brain, which may be strongly sex-dependent, remains poorly understood.

Air pollution is a complex mixture of particulate matter (PM) and gases. PM exists in various size fractions ranging from ultrafine (particle diameter $D_p < 100$ nm; $PM_{0.1}$) to fine ($D_p < 2.5$ μ m; $PM_{2.5}$) and coarse mode (2.5 μ m $< D_p < 10$ μ m; PM_{10}), and it is composed of myriad chemical constituents like metals, inorganic salts, black carbon, and organic compounds. Traffic-related air pollution (TRAP) is one of the most well-characterized and frequently encountered types of air pollution in urban areas, especially by those who live or work near roadways and experience daily commuter or drayage traffic.¹³ TRAP is a mixture of PM and gases emitted from gasoline-powered light-duty vehicles (LDV), diesel-powered heavy-duty vehicles (HDV), non-combustion derived tire, brake and engine wear, and resuspended road dust, which all vary widely in physiochemical composition. TRAP-derived gases include nitrogen oxides (NO_x), sulfur dioxide (SO_2), volatile and semi-volatile organic compounds (VOCs/SVOCs) like benzene and polycyclic aromatic hydrocarbons (PAHs), and carbon monoxide (CO). Due to the heterogenous nature of TRAP, the contributions of specific TRAP components to biological outcomes remain unclear. Many paradigms tested in experimental studies exposed animals to variations of concentrated PM generated in the laboratory or by concentrated ambient particle (CAP)⁹ systems, which collect and concentrate real-world PM and may exclude or change gaseous vapor compositions. While these paradigms allow investigation of exposures to high concentrations of single sources or components, the importance of using more real-world-representative mixtures of air pollutants^{10,11,14} has grown as air pollution dynamics rapidly shift due to changing weather and climate patterns, altered vehicle fleet mixtures, traffic patterns and fuel formulations, and new emission control technologies and regulatory policies, as well as the presence of co-stressors like noise and vibration. Moreover, studies have shown that chronic exposures to ambient levels of air pollution can trigger biological responses that differ from acute exposures (reviewed in³).

To address these data gaps, we exposed TgF344-AD rats, a model that expresses human risk genes for early onset AD, to

realistic temporal dynamics, duration, concentrations, and compositions of TRAP that humans experience in near-roadway environments and during commutes.¹⁵ Building on a previous study that showed the strongest phenotypes of TRAP effects on the time-to-onset and overall burden of amyloid plaques and neuronal cell loss in TgF344-AD rats¹⁰ but not in wild-type rats, we aimed to examine the contributions of different TRAP components to pulmonary and systemic inflammatory responses, which may promote neuroinflammation and AD pathogenesis via the lung–brain axis. Because wild-type rats do not develop amyloid pathologies, a distinguishing clinical hallmark of AD, even in response to TRAP, we focused on the TgF344-AD rats in this study. Specifically, we compared the combined and separate impacts of PM and gases from LDV or a mix of LDV + HDV exhausts by leveraging the Caldecott Tunnel Exposure Facility (CTEF), which allows real-world exposure to traffic emissions directly drawn from a major highway tunnel. In addition, we investigated the sensitivity of peripheral inflammatory responses to different TRAP components by sex. We hypothesized that the combined exposure to a broad mixture of all the TRAP components (PM + gas from LDV + HDV) induces the most severe and synchronous inflammation across the respiratory and circulatory systems, demonstrating synergistic or additive effects of PM and gas constituents derived from mixed traffic including gasoline and diesel emissions. Identifying how different TRAP components distinctly drive specific peripheral inflammation can aid in the implementation of more targeted health interventions and community-level prevention to reduce both neurological and respiratory health burdens in susceptible populations.

METHODS

Animal Breeding

All animal procedures followed the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines and were approved by the University of California, Davis (UC Davis) Institutional Animal Care and Use Committee (IACUC, protocol #23921). Transgenic TgF344-AD rats that express human mutant amyloid precursor protein (APPSW) and mutant presenilin 1 (PS1 Δ E9) on a Fischer-344 strain background were bred at UC Davis as previously described.¹⁰ Briefly, hemizygous TgF344-AD males were bred with Fischer-344 wild-type females. Newborn pups were marked with unique tattoos on their paws using an animal tattoo ink (329AA, Ketchum Manufacturing Inc.) between postnatal day (PND) 2–4, tail-clipped between PND 7–10 for genotyping, and weaned on PND 21. A total of 976 wild-type and transgenic animals were generated for the original study, encompassing exposure durations of 3, 8, 11, and 14 months, of which 432 TgF344-AD rats with confirmed transgenic genotypes were selected for the study. At each timepoint, a subset of 108 TgF344-AD rats (54 females and 54 males) was collected and

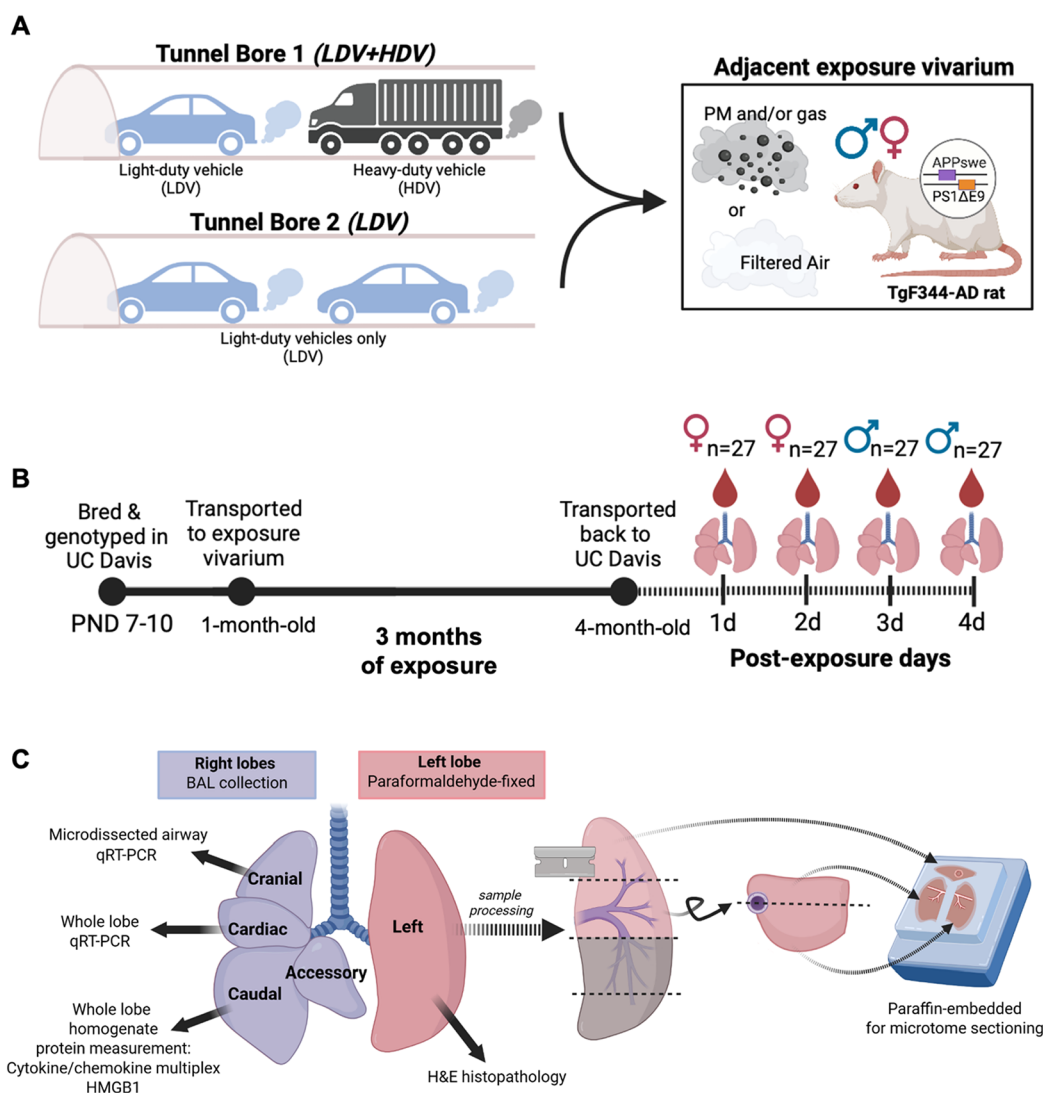


Figure 1. Experimental timeline, exposure paradigm, and lung sample processing workflow. (A,B) Male and female TgF344-AD rats were bred and genotyped at UC Davis between postnatal days (PND) 7–10, then transported to the Caldecott Tunnel Exposure Facility (CTEF) at 1 month of age. At the exposure facility, animals were randomly divided into one of the 6 exposure groups: filtered air (FA) control, gas only, particulate matter (PM) only, or both gas and PM. These emissions were collected from tunnel bore 1 that allowed both light- and heavy-duty vehicles (LDV + HDV) or tunnel bore 2 that allowed LDV only and animals received continuous exposure while in the exposure facility. After 3 months of exposure, animals were brought back to UC Davis where lungs and blood were collected at 1-, 2-, 3-, or 4 days (d) post-exposure. (C) During necropsy, right and left lung lobes were ligated and separated for different end points, including bronchoalveolar lavage (BAL) fluid collection, gene and protein extractions, and histopathology. Paraformaldehyde-fixed left lung lobes were further dissected into 3 portions to expose the airway for H&E histopathology assessment.

analyzed. This report describes data generated from the subset of animals exposed for 3 months of duration.

Animal Exposure

On PND 28, female and male TgF344-AD rats were transported to the CTEF, an IACUC-approved vivarium and exposure facility immediately adjacent to a major freeway tunnel in Oakland, California, USA. As previously described,^{10,16} TRAP emissions were directly drawn from the tunnel bores and delivered in real-time to the rats housed in whole-body exposure chambers. Emissions from tunnel bore 1, which allows both LDV + HDV, or bore 2, which is open to only LDV, were delivered to rats unfractionated (PM + Gas) or fractionated into PM only or gas only prior to being delivered to exposure chambers. Control animals were also housed at the CTEF, and thus experienced the same levels of traffic noise and vibration, but these animals were exposed to filtered air (FA) delivered to the exposure chamber after removal of VOCs and PM using activated carbon and ultrahigh-efficiency particle filtration, respectively.¹⁶

Animals were randomly assigned to one of the 6 different exposure groups (Table 1) using a Microsoft Excel (Microsoft Corporation; Redmond, WA, USA) random number generator. The exposure chambers in the CTEF were controlled to maintain temperature at 20–26 °C, and animals were exposed to a 12:12 (h/h) light–dark cycle. Same sex animals within the same exposure group were housed 3 per cage with food (2018 Teklad Global 18% Protein Rodent Diets, Envigo Global Services, Inc.; Denver, PA, USA) and filtered water provided ad libitum. Daily animal monitoring and weekly cage changes were performed, and daily levels of humidity, temperature, traffic volume, PM concentration, and noise in the exposure vivarium were monitored and recorded. The initial sample size for exposure was $n = 9$ per exposure group per sex, and $n = 4–9$ after sample processing and exclusion of statistical outliers. The N is indicated in each figure legend.

Characterization of PM in TRAP

PM in bore 1 (LDV + HDV) and bore 2 (LDV) of the tunnel was measured as previously described.¹⁶ Briefly, real-time particle mass concentrations and size distributions of PM_{0.3}, PM₁, PM_{2.5}, PM₄, PM₁₀, and total suspended particle (TSP) were continuously measured every 1 s (s) using an optical particle counter (DustTrak model 8533 DRX; TSI Inc.; Shoreview, MN, USA) and every 3 min (min) using a scanning mobility particle sizer (model 3938; TSI Inc.; Shoreview, MN, USA), respectively. While data for each metric were pooled across the final 11 months of the original 14 month study period, the data are representative of the study period within the reported variances based on historical trends from data collected at the CTEF over the last eight years.^{10,11,16}

Endotoxin levels in extracts of PM filter samples were measured using a Pierce Chromogenic Endotoxin Quant Kit (A39552, Thermo Fisher Scientific; Carlsbad, CA, USA) following the manufacturer's instructions (see the [Supporting Information Methods](#) for assay details). All procedures were performed using sterile materials and standard aseptic techniques. Measured endotoxin concentrations (EU/mL) were normalized to the total volume of air sampled through the filters and then corrected for the extraction efficiency, defined as the mass of PM extracted for analysis relative to the total mass of PM sampled, to calculate the final airborne endotoxin concentration reported in the unit of EU/m³.

Animal Necropsy. After 3 months of exposure, animals ($n = 108$) were transported back to UC Davis and habituated in an animal holding room overnight prior to necropsy. Due to logistical challenges of euthanizing all the animals at one time, animal necropsy was spread across 4 days (d) post-exposure. Females were euthanized over 1 and 2 d post-exposure; males, over 3 and 4 d post-exposure ([Figure 1](#)). Same-sex animals from different exposure groups were distributed across the two different collection days. Animals were anesthetized with aerosolized 5% isoflurane (Isospire, Dechra; Northwich, United Kingdom) in oxygen delivered at a rate of 1.5 L/min. Whole blood was collected via cardiac puncture into EDTA-plasma tubes (366643, BD Vacutainer; Broken Bow, NE, USA) and SST-serum tubes (367989, BD Vacutainer; Broken Bow, NE, USA). For plasma, whole blood in EDTA-plasma tubes was centrifuged at 2000g for 15 min at 4 °C, and for serum, whole blood in SST-serum tubes was centrifuged at the same setting after 30 min incubation at room temperature. Rats were transcardially perfused with 200 mL of cold 0.1 mol/L phosphate-buffered saline (PBS; pH 7.2) at a rate of 15 mL/min using a Masterflex peristaltic pump (Cole Parmer; Vernon Hills, IL, USA) to clear the vasculature in the brain. After perfusion, the trachea was cannulated, and all the lung lobes were harvested from the body. Bronchoalveolar lavage (BAL) was collected from the right lobes after ligating the left lobe by instilling 5 mL of PBS (97062-818, VWR; Radnor, PA, USA) 4 times to fully inflate the right lobes ([Figure 1C](#)). Recovered BAL fluid was centrifuged at 800g for 15 min at 4 °C for cytospin preparation. Then, cranial and cardiac lung lobes were instilled with 20 mL of RNAlater (AM8021, Thermo Fisher-Invitrogen; Carlsbad, CA, USA) and stored at −20 °C until RNA extraction. Lastly, caudal and accessory lobes were flash frozen in liquid nitrogen and stored at −80 °C until tissue homogenization ([Figure 1C](#)). The ligated left lobe was separated, cannulated, and inflated with 1% (w/v) paraformaldehyde (Thermo Fisher Scientific; Carlsbad, CA, USA) fixative dissolved in 0.1 mol/L PBS (pH 7.2) at a constant pressure using a manometer at a 30 cm height for either 1 h or until 100 mL of fixative had flowed through. Left lobes were further immersed in 1% paraformaldehyde for 24 h until dehydration and paraffin-embedding.

Lung Tissue Homogenization. Using a sonicator (Virtis Virsonic, SP Industries Inc.; Stone Ridge, NY, USA), flash-frozen caudal lung lobes were homogenized in T-PER tissue protein extraction buffer (78510, Thermo Fisher Scientific; Carlsbad, CA, USA) with Halt protease and phosphatase inhibitor cocktail (78446, Thermo Fisher Scientific; Carlsbad, CA, USA) at a ratio of 0.1 g/mL. Tissue homogenates were centrifuged twice at 10,000g for 10 min at 4 °C to collect clear supernatant. The total protein concentration of the

homogenate supernatant was measured using a Pierce bicinchoninic acid assay (23225, Thermo Fisher Scientific; Carlsbad, CA, USA).

Cytokine and Chemokine Multiplex Assay. Plasma samples and caudal lung homogenates were analyzed using a cytokine multiplex assay. Caudal lung homogenates were normalized to 2 mg/mL total protein concentration, and plasma samples were diluted 2-fold with PBS (97062-818, VWR; Radnor, PA, USA). All samples were shipped on dry ice to Eve Technologies Corp. (Calgary, AB, Canada) to measure 27 cytokines simultaneously in their Rat Cytokine 27-Plex Discovery Assay® (MilliporeSigma; Burlington, MA, USA) on a Luminex 200 system (Luminex; Austin, TX, USA). Observed and final concentrations were extrapolated from point-to-point semi-log regression standard curves for each cytokine. All samples were analyzed in two technical replicates, which were averaged for the final statistical analysis. Gene ontology pathway analyses were performed using the STRING database¹⁷ for cytokines and chemokines that were elevated in the plasma or lung in each TRAP group relative to sex-matched FA controls using a relaxed threshold of p adj < 0.1 for exploratory analysis. STRING functional enrichment results ([Tables S3–S6 and S8](#)) were then filtered to terms with FDR < 0.05 and ranked by log₁₀ fold enrichment, FDR, and observed gene count to prioritize more targeted pathways. The top 15 enriched pathways were visualized using RStudio software (R version 4.4.1, Posit; Boston, MA, USA).

Lung RNA Extraction and qRT-PCR

Whole cardiac lobes preserved in RNAlater were homogenized with steel beads (SSB14B, Next Advance; Troy, NY, USA) on a bullet blender (Next Advance; Troy, NY, USA) to extract RNA. RNA was extracted using the RNeasy Plus Mini Kit (74136, Qiagen; Venlo, Netherlands), and a total of 2.1 μg of RNA was reverse-transcribed to cDNA using a High-Capacity cDNA Reverse Transcription kit (4368814, Thermo Fisher-Applied Biosystems; Carlsbad, CA, USA). Reverse-transcribed cDNA was diluted, and a final concentration of 3 ng/μL was loaded on the qRT-PCR plate with the Taqman Fast Universal Master Mix reagent (4364103, Thermo Fisher-Applied Biosystems; Carlsbad, CA, USA) and Taqman primers for each gene of interest (see the [Supporting Information Methods](#) for primer details). Three reference genes were selected based on published studies that identified airway housekeeping genes with stable expression in rodent models of pulmonary inflammation¹⁸ and fibrosis.¹⁹ Each sample's genes of interest and reference genes were run on the same qRT-PCR plate on QuantStudio6 Flex Real-Time PCR Systems (Thermo Fisher-Applied Biosystems; Carlsbad, CA, USA).

For qRT-PCR analyses of different airway regions, RNAlater-preserved trachea and cranial lobe was microdissected into proximal airway, distal airway, and parenchyma following the same extraction and cDNA synthesis steps described above. Relative log₂ fold-change expressions of normalized genes of interest were calculated using Livak's method.²⁰

High Mobility Group Box 1 ELISA

High mobility group box 1 (HMGB1) concentrations were measured in the serum and caudal lung lobe homogenates using a commercial ELISA (NPB3-06661, Novus Biologicals; Centennial, CO, USA) following the manufacturer's instructions (see the [Supporting Information Methods](#) for assay details).

Bronchoalveolar Lavage Cytospin Differential Staining. On the day of necropsy, BAL was centrifuged for separation into the supernatant and cell pellets. The separated cell pellets were resuspended in 1.5–3.0 mL (to dilute contaminating red blood cells) of Dulbecco PBS (D8537-500ML, Sigma-Aldrich; St. Louis, MO, USA). The resuspension volumes for each animal were noted. Total viable and nonviable cells in the pellet resuspension were counted using a hemacytometer and trypan blue (T8153-100ML, Sigma-Aldrich; St. Louis, MO, USA). Normalized concentrations of cells were loaded on a microscope slide using cytospin centrifugation (Shandon/Fisher Scientific; Waltham, MA, USA). BAL cytospins were further fixed and differentially stained with Hema-Diff (STL1198516, STL1197516, STL1198016, StatLab; McKinney, TX,

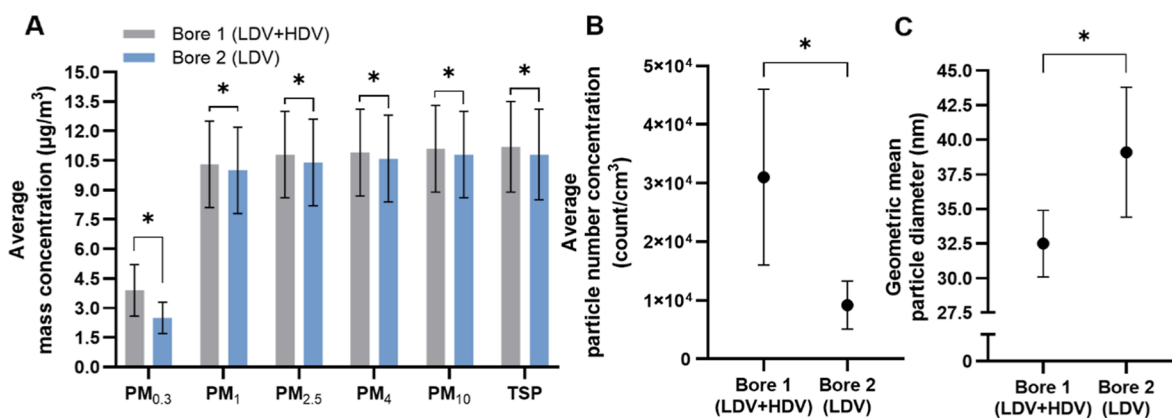


Figure 2. Characterization of PM in tunnel bores. (A) Average PM_{0.3}, PM₁, PM_{2.5}, PM₄, and PM₁₀ and total suspended particle (TSP) mass concentrations $\pm$ SD, (B) average TSP number concentrations $\pm$ SD, and (C) geometric mean particle diameter $\pm$ SD for bore 1 (LDV + HDV) and bore 2 (LDV). Real-time particle mass and number concentrations were continuously measured every 1 s, while size distributions were measured every 3 min. Data for each metric were pooled across the final 11 months of the original 14 month study period and analyzed with Welch's *t*-test and Tukey–Kramer HSD to identify significant differences between bore 1 (LDV + HDV) and bore 2 (LDV); **p* adj < 0.05, †*p* adj < 0.1.

USA). For differential cell counting, a single experimenter blinded to group counted cells based on standard morphological criteria used to identify macrophages, neutrophils, lymphocytes, and eosinophils. The final number of each immune cell type in BAL was calculated on the basis of the percentage of cell type, total cell concentration, recovered BAL volume, and BAL pellet resuspension volume.

Left Lung Lobe Histopathology. Paraformaldehyde-fixed left lung lobes were cut for embedment as 3 portions (Figure 1C) to expose the branching of the airway for pathology and transferred to 70% (v/v) ethanol after 24 h immersion in 1% paraformaldehyde. Serial dehydration in 70%, 80%, 95%, 100% ethanol, 50% toluene and 50% ethanol solution, and 100% toluene was performed using an autotechnicon (Technicon Corp; Greensboro, NC, USA). All ethanol working solutions were made with 190 and 200 proof KOPTEC ethanol (89125-188, 89125-166, VWR; Radnor, PA, USA) diluted in ultrapure water. After dehydration, lung tissue was embedded in paraffin (39603002, Leica Biosystems; Nussloch, Germany) for microtome (Leica Microsystems; Wetzlar, Germany) sectioning at 3–4 μ m thickness. The paraffin sections were stained with hematoxylin (GHS316-500ML, Sigma-Aldrich; St. Louis, MO, USA) and eosin (E511-2S, Fisher Scientific; Waltham, MA, USA) (H&E). For histopathologic assessment, H&E staining was semi-quantitatively scored by a board-certified veterinary pathologist for two end points based on the observations of: (1) abundance of bronchus-associated lymphoid tissue (BALT) formed by aggregates of leukocytes and (2) thickening or expansion of airway walls as an indication of inflammation. BALT formation was scored on a 0–4 scale based on the volume of aggregates in the large, medium, small, and pulmonary vessels near terminal airways deep in the parenchyma (0 = no aggregates; 4 = large increase in aggregates). Airways were classified by size and generations of branching, from the main bronchus to terminal bronchioles immediately before entering the alveolar parenchyma. BALT scores from the large and medium airways were summed as the upper airway region, while scores from the small and terminal airways were summed as the lower airway region. Inflammation was scored using a binary system with 0 or 1 assigned based on either absence or presence of tissue thickening across all airways. All representative images were taken with a BH2 microscope (Olympus Corp.; Tokyo, Japan) at 4 $\times$ and 20 $\times$ magnifications and were organized using Fiji software (ImageJ version 1.54f, National Institutes of Health; Bethesda, MD, USA).

Statistical Analysis. Linear regression models were fit for all experimental outcomes to compare each TRAP exposure group to the sex- and age-matched FA control group. Female and male data were analyzed separately given known underlying biological sex differences in airway anatomy,²¹ inflammation,²² and AD pathogenesis²³ and

because females and males were euthanized on different postexposure days. Because sex and postexposure day were confounded, we limited our analysis to comparisons of TRAP groups with sex-matched FA controls within each sex rather than between sexes. To account for differential responses due to animal euthanasia on two different postexposure days within each sex, postexposure day was included as a factor for regression models along with the exposure group, which was our primary factor of interest. For each outcome, interaction between exposure group and postexposure day was considered and compared against a no-interaction (main effects model) with corrected Akaike Information Criterion (AICc)²⁴ ranking to select the best-fitting statistical model. On the basis of the AICc results, a main effects regression model without interaction was selected for all outcomes, which indicates that there was no significant dependency between the exposure group and postexposure day. Yet, postexposure day was not ignored and was accounted for in the analysis of each outcome by including it in the main effects regression model, which predicted the effects of exposure group averaged over postexposure days.²⁵

Key PM measurements between the two tunnel bores (bore 1: LDV+HDV and bore 2: LDV) were analyzed using Welch's *t*-test with Tukey–Kramer HSD adjustment to account for multiple comparisons via statistical software (JMP Pro 17, JMP Statistical Discovery LLC; Cary, NC, USA).

Based on the expected effect size, variability, and tissue availability, initial sample sizes varied by outcome, with either 6 or 9 animals per group per sex. Values outside ± 1.5 times the interquartile range²⁶ of each group per sex were identified as outliers and excluded before linear regression analysis for each outcome, provided that the final group sample size remained at least 4. Data were transformed to log₂ scale after adding a small constant to improve data normality and fit for linear regression. Normality and residuals of regression models were assessed with quantile–quantile and residual plots. Following linear regression, estimated marginal means²⁷ was used to construct contrasts between TRAP exposure groups and sex-matched FA control with a standard cluster-robust variance-covariance sandwich estimator²⁸ to correct for heteroskedasticity and potential clustering of outcomes that may have arisen from repeated measurements taken from the same sets of animals. Then, post hoc Benjamini-Hochberg FDR correction was applied to the unadjusted *p*-values from contrasts across all cytokines to account for multiple testing in the 27-plex cytokine panel. For all other outcomes, Dunnett's adjustment for multiple comparison was applied. Adjusted *p*-values (*p* adj) are indicated in each figure as **p* adj < 0.05, †*p* adj < 0.1. All raw statistical results are included in the supplemental tables (Tables S1–S15). For pairwise correlational analyses, Pearson's correlation with Benjamini-Hochberg FDR correction was performed for quantitative

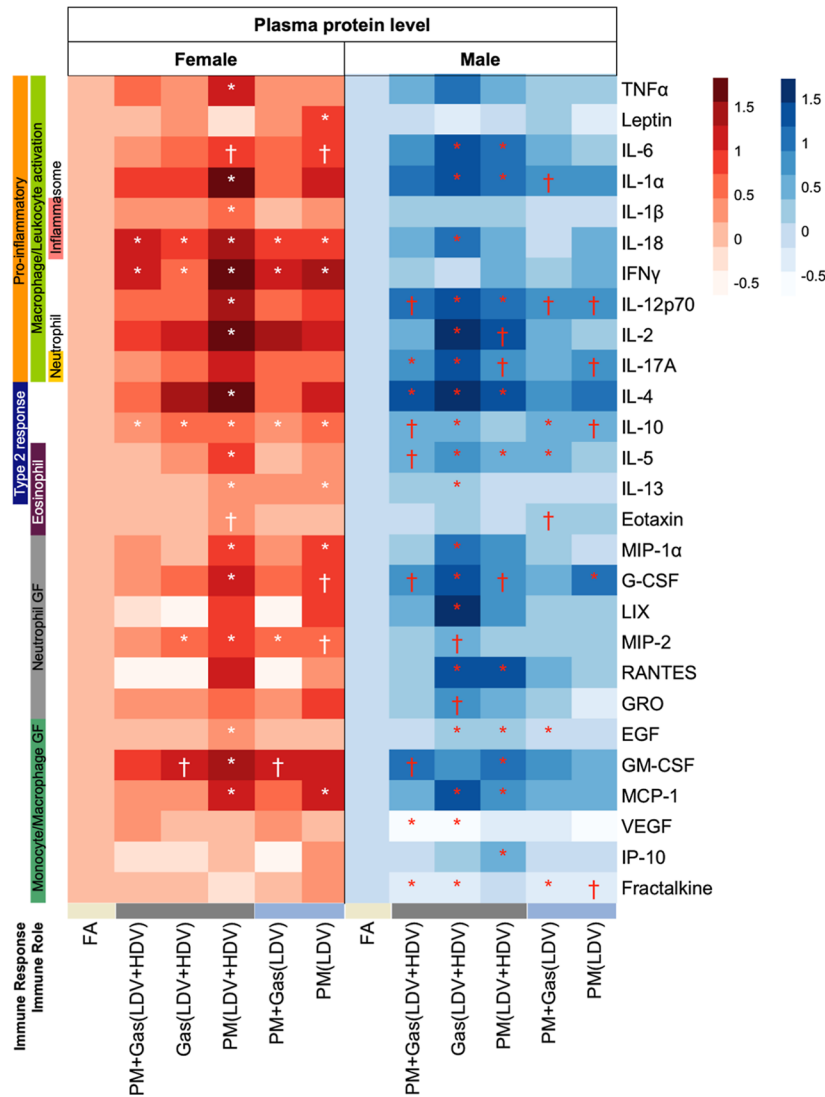


Figure 3. Sex-specific effects of TRAP on plasma cytokine and chemokine protein levels in 4 month-old TgF344-AD rats after 3 months of exposure. Data were analyzed using linear regression, followed by contrasts between TRAP groups and FA control. To account for multiple testing across the cytokine panel, unadjusted *p*-values from comparisons across all cytokines were corrected using the Benjamini-Hochberg false discovery rate; **p*. adj < 0.05, †*p*. adj < 0.1. Female and male data are presented as a log₂ fold-change of the exposure group mean normalized to the sex- and age-matched FA control group mean. Sample size per group per sex ranged from *n* = 4–7 across the cytokines after exclusion of statistical outliers.

outcomes within each exposure group for females and males separately. All statistical analyses other than particle characterization were performed using RStudio software (R version 4.4.1, Posit; Boston, MA, USA).

RESULTS

Differential Particle and Gas Phase Concentrations

To quantify differences between LDV + HDV and LDV-only particle emissions, the size distribution of average particle number concentrations of PM_{0.3}, PM₁, PM_{2.5}, PM₄, and PM₁₀, average TSP mass concentrations, and geometric particle diameters (Figure 2) were measured independently for each tunnel bore. The inter-bore differences in study-long average PM mass concentrations were small in magnitude but statistically significant; PM concentrations in LDV + HDV averaged 0.52 μg/m³ higher than those in LDV-only across all size fractions. Specifically, the average PM_{2.5} mass concentration was 0.4 μg/m³ higher in LDV + HDV (10.8 μg/m³) than in LDV-only (10.4 μg/m³) (Figure 2A and Table S1).

However, the differences in average particle number concentration were striking with LDV + HDV a factor of ~3.4 times higher, on average, than LDV-only (Figure 2B and Table S1). To put this in the perspective of the PM mass comparison, PM_{0.3} (*D_p* < 300 nm) mass concentration, which was obtained by integrating the particle size distributions and converting to mass assuming spherical particles with density = 1.2 g/mL, was higher, on average, by 1.4 μg/m³ in the LDV + HDV bore, which is substantial considering that particle mass scales as the cube of particle diameter and this inter-bore difference is for the smallest particle sizes (i.e., <300 nm versus 1–10 μm). These trends are consistent with the fact that LDVs and HDVs predominantly emit ultrafine particles, which is also reflected by a ~20% smaller geometric mean particle diameter measurement in LDV + HDV than in LDV-only (Figure 2C). Collectively, ultrafine particles from LDV + HDV contribute negligible mass to the atmosphere due to their small size (<100 nm) so that PM mass concentrations at CTEF are dominated by the background airshed, which is common to both bores.

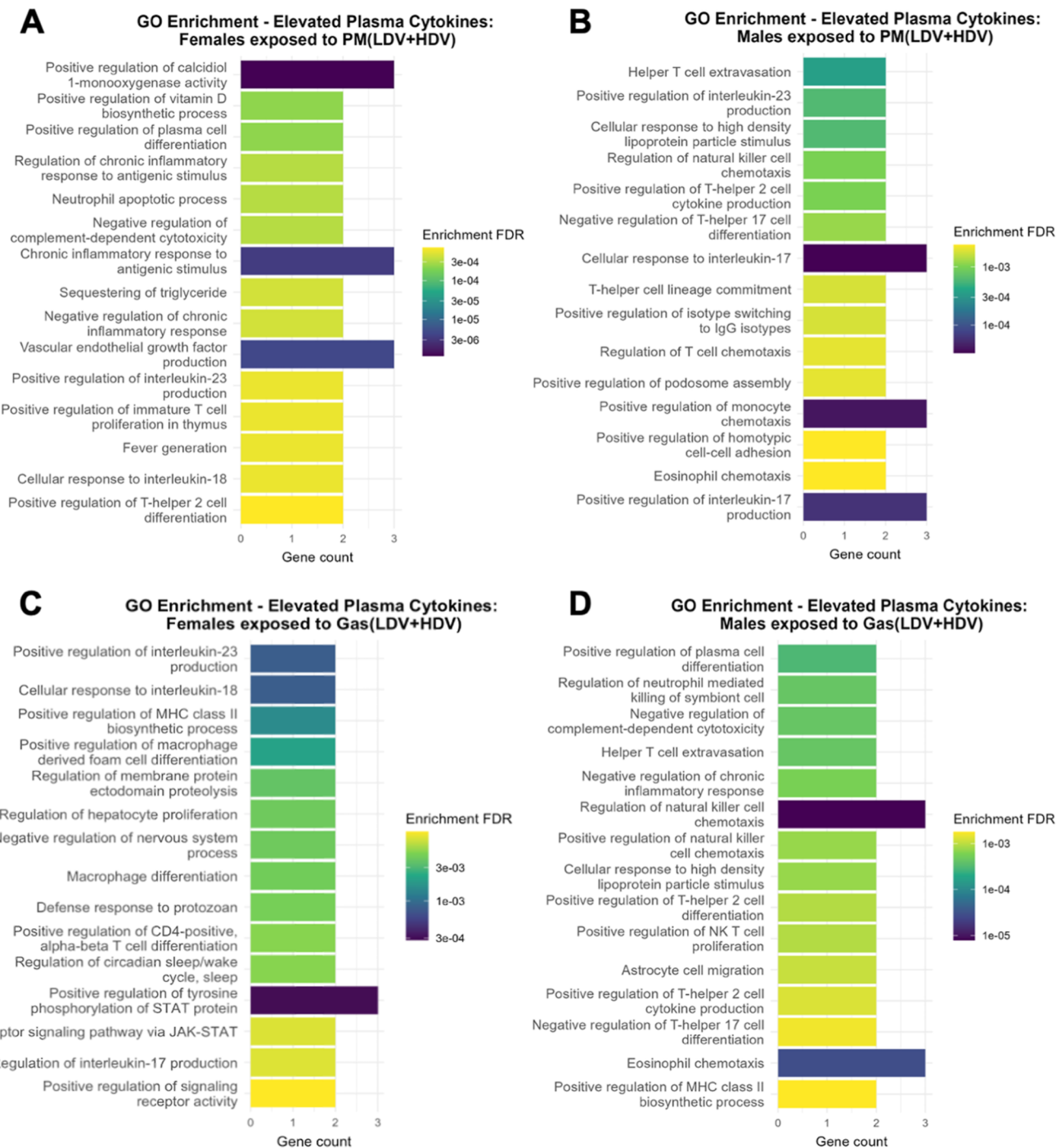


Figure 4. Gene ontology (GO) enrichment pathway analyses plasma cytokines that were significantly ($p. adj < 0.1$) elevated in 4 month-old TgF344-AD rats after 3 months of exposure. (A) Females exposed to PM(LDV + HDV), (B) males exposed to PM(LDV + HDV), (C) females exposed to Gas(LDV + HDV), and (D) males exposed to Gas(LDV + HDV). Pathway analyses were performed using enrichment results from the STRING database platform, top 15 enriched pathways ranked by $\log_{10}$ fold enrichment, FDR, and observed gene counts.

To examine the contribution of endotoxin to respiratory and systemic inflammatory responses,^{29,30} we measured LPS concentrations in PM filter samples collected at CTEF. The study-long average endotoxin concentration was $3.17 \times 10^{-4} \pm 1.52 \times 10^{-4}$ EU/m³.

Component-Dependent TRAP Effects on Circulating Cytokine Concentrations in Females and Males across Different Post-Exposure Days

To evaluate TRAP effects on systemic inflammation, a multiplex assay was used to measure plasma protein concentrations of 27 cytokines and chemokines, some of which have previously been shown to be elevated with air pollution exposure in humans.³¹ Females had strong systemic type 1 inflammatory responses after 3 months of exposure to all types of TRAP emission components compared to sex- and

age-matched FA controls (Figure 3). While IFN- γ , IL-18, and IL-10 were significantly elevated in all TRAP groups, the most robust increases were observed in the PM(LDV + HDV) group: 3.1-, 2.6-, and 1.6-fold increases, respectively (Table S2). Increased type 1 (TNF- α , IL-1 β , IL-1 α , IL-6, IL-2) and type 2 cytokines (IL-4, IL-5, IL-13) and chemokines (eotaxin, MIP-1 α , G-CSF, MIP-2, EGF, GM-CSF, and MCP-1) were observed only in the females exposed to PM(LDV + HDV). In contrast, TRAP did not induce strong systemic type 1 responses in males following the same exposure. While Th1-promoting IL-6, IL-12p70, IL-2, and IL-1 α were increased in males exposed to LDV+HDV, there were no significant changes in key Th1-inducing IFN- γ and IL-1 β , in any of the TRAP groups. Instead, Th2 responses (IL-4, IL-5, and IL-13), Th17 responses (IL-17A, IL-6), and neutrophil recruitment/growth factors (MIP-1 α , LIX, MIP-2, GRO, EGF, G-CSF, LIX, RANTES) were elevated most robustly in the Gas(LDV + HDV) group. An additional difference between exposure-matched females and males was that VEGF and fractalkine macrophage recruitment/growth factors were downregulated in Gas(LDV + HDV)-exposed males but not in females.

Pathway analysis of elevated cytokines and chemokines (p adj < 0.1) (Figure 4) revealed that females exposed to PM(LDV + HDV) showed increased pro-inflammatory signaling associated with regulations of plasma cell differentiation, antigenic responses, T-cell proliferation, fever generation, IL-18 responses, along with compensatory resolution of inflammation, including calcidiol 1-monooxygenase activity, vitamin D biosynthesis, vascular endothelial growth factor production, Th2 cell differentiation, and negative regulations of complement-dependent cytotoxicity and chronic inflammatory responses (Figure 4A, Table S3). However, males exposed to the same PM(LDV + HDV) exposure had different immune pathways upregulated, including pathways involved in regulation of Th2 and Th17 differentiations, IL-17 responses, IgG isotype switching, and chemotaxis of T cells, eosinophils, monocytes, and natural killer (NK) cells (Figure 4B, Table S4). Gas(LDV + HDV) exposure promoted macrophage-activating immune pathways in females, including macrophage differentiation, MHC class II biosynthesis, JAK-STAT receptor signaling pathway, and regulation of responses to IL-23, IL-18, and IL17 (Figure 4C, Table S5). For males exposed to Gas(LDV + HDV), which caused the greatest number of significant increases in the plasma cytokines among other TRAP groups, the immune signaling pathways were similar to those of the sex-matched PM(LDV + HDV) group and included signaling pathways involved in regulation of Th2 and Th17, plasma cell differentiation, neutrophil-mediated cytotoxicity, chemotaxis of T cells, eosinophils, and NK cells and negative regulation of complement-dependent cytotoxicity (Figure 4D, Table S6).

Robust Sex-Biased TRAP Effects on Cytokine Concentrations in the Lung Homogenate across Different Post-Exposure Days

Protein concentrations of the same array of cytokines and chemokines measured in plasma were also measured in lung tissue homogenates to assess pulmonary inflammatory responses. In contrast to the female plasma cytokine data (Figure 3), most changes in the lung cytokines were statistically insignificant in females (Figure 5). While leptin levels were significantly increased by 1.5- to 1.6-fold (Table S7) in the lung of all TRAP groups, the only other significant

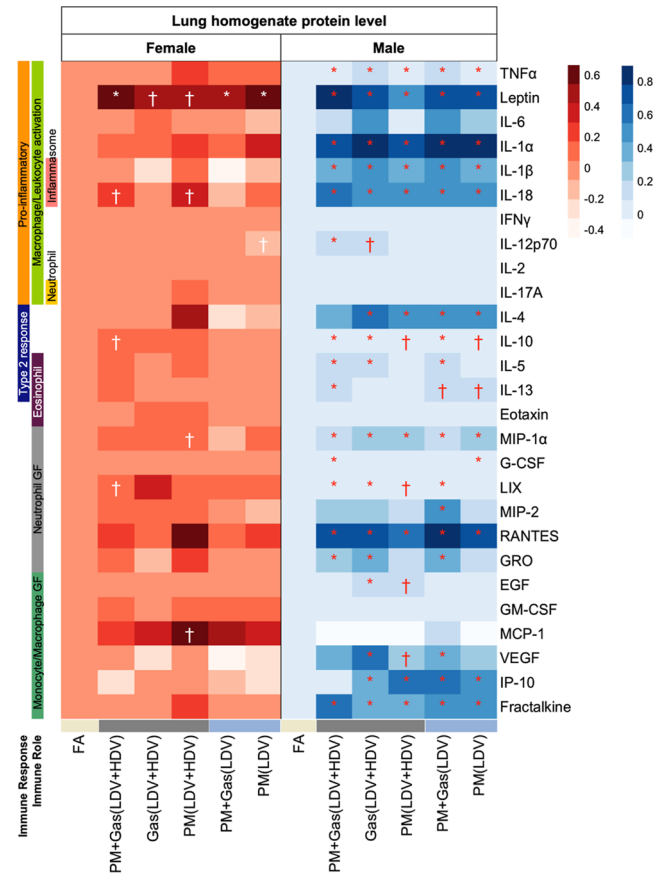


Figure 5. Sex-specific effects of TRAP on caudal lung lobe tissue homogenates. Cytokine and chemokine protein levels were measured in 4 month-old TgF344-AD rats after 3 months of exposure. Data were analyzed using linear regression, followed by contrasts between TRAP groups and FA control. To account for multiple testing across all cytokine panel, unadjusted p -values from comparisons across all cytokines were corrected using the Benjamini-Hochberg false discovery rate; * p . adj < 0.05, † p . adj < 0.1. Female and male data are presented as a log₂ fold-change of exposure group mean normalized to sex- and age-matched FA control group mean. Sample size per group per sex ranged from $n = 4$ –7 across the cytokines after exclusion of statistical outliers.

increases in lung cytokines were observed in PM(LDV + HDV), with elevated levels of IL-18, MIP-1 α , MCP-1, and in PM+Gas(LDV+HDV), with elevated levels of IL-18, IL-10, and LIX (Figure 5). However, TRAP caused substantial changes in male lung cytokine profiles when compared to age- and sex-matched FA controls. There were significant increases in pro-inflammatory TNF- α and IL-1 family cytokines (IL-1 α , IL-1 β , IL-18) involved in type 1 responses as well as injury responses, and in anti-inflammatory cytokines (IL-4, IL10) involved in type 2 responses across all TRAP groups in male lungs (Figure 5). An additional difference between females and males was observed in increased RANTES and fractalkine, which function as leukocyte recruitment factors, across all TRAP exposures. When commonly increased cytokines and chemokines in male lungs across all TRAP groups were analyzed, strong pro-inflammatory response pathways were identified (Figure S1 and Table S8).

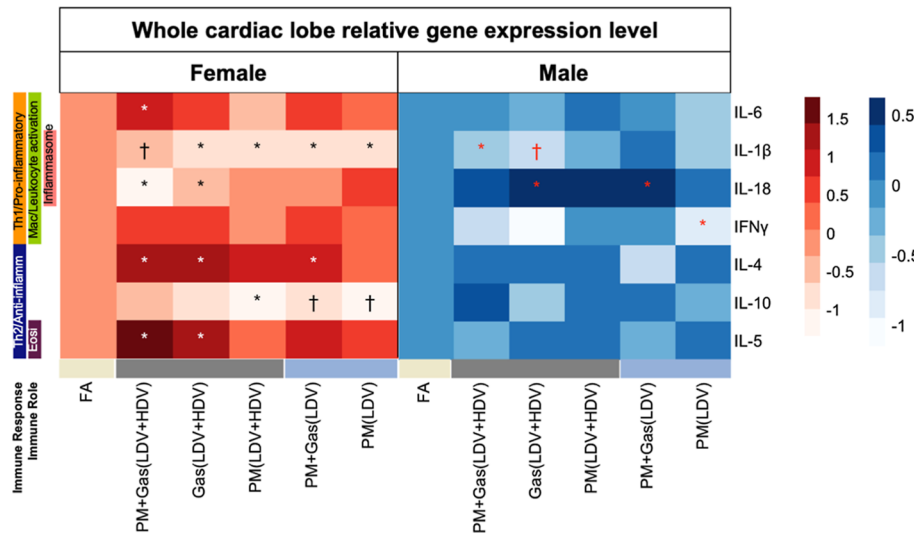


Figure 6. Sex-specific effects of TRAP on relative cytokine gene expression levels in 4 month-old TgF344-AD rats after 3 months of exposure in whole cardiac lung lobe. Data were analyzed using linear regression, followed by contrasts between TRAP groups and FA control with Dunnett's adjustment for multiple comparisons; **p*. adj < 0.05, †*p*. adj < 0.1. Female and male data are presented as a log₂ fold-change of exposure group mean normalized to sex- and age-matched FA control group mean. Sample size per group per sex ranged from *n* = 4–7 across the cytokines after statistical outlier exclusion.

Regulation of Cytokine Gene Transcription in the Lung in Response to TRAP

To further examine TRAP-induced changes in pro- and anti-inflammatory cytokines at the transcription level in the lung, we quantified relative gene expressions of key Th1 and Th2 cytokines in the whole cardiac lung lobe (Figure 6). Interestingly, TRAP-induced changes observed in cytokine protein concentrations were not reflected in gene expression in the lung. In the male lung, there were significant changes in protein levels of IL-1β, IL-18, IL-4, IL-10, and IL-5 across most TRAP exposures (Figure 5). However, there were no significant changes in gene expression of these cytokines except in IL-18 transcript, which was 1.5-fold upregulated in both the Gas(LDV + HDV) and PM + Gas(LDV) groups, in IFN-γ transcript that was downregulated by 2-fold in PM(LDV), and in IL-1β transcript that was downregulated by 1.45-fold in PM + Gas(LDV + HDV) and Gas(LDV + HDV) (Figure 6 and Table S9). On the other hand, in the female lung, there were significant changes observed in gene expression of both Th1 (IL-6, IL-1β, IL-18) and Th2 (IL-4, IL-10, IL-5) cytokines across different TRAP groups, which were not observed at the protein level (Figure 5). Most gene expression of Th1 cytokines in females was downregulated, with 1.4- to 2-fold decreases in IL-1β transcript across all TRAP groups and 1.4- to 1.8-fold decreases in IL-18 transcript in PM + Gas(LDV + HDV) and Gas(LDV + HDV). Th2 cytokines were upregulated in females. Gene expression of IL-5 increased in PM + Gas(LDV + HDV) and Gas(LDV + HDV) by 3.4- and 2.2-fold, respectively; and gene expression of IL-4 was increased in PM + Gas(LDV + HDV), Gas(LDV + HDV), and PM + Gas(LDV) by 2.7-, 2.4-, and 1.9-fold, respectively. While most of the Th1 cytokines were generally downregulated, IL-6 expression was upregulated by 1.8-fold in PM + Gas(LDV + HDV). Similarly, unlike other Th2 cytokines that were generally upregulated, IL-10 expression was downregulated by 2.5-, 2.38-, 1.85-fold in PM(LDV + HDV), PM(LDV), and PM + Gas(LDV), respectively (Figure 6 and Table S9).

Airway Region-Dependent Changes in Cytokine Gene Expressions

Given known differences in the cell types and airway deposition of PM and gases,³² it is possible that deposition of exposure components may impact specific lung regions differentially. We tested this by assessing key type 1 and type 2 cytokine gene expression in trachea and microdissected airways of proximal airway, distal airway, and parenchyma. Region-dependent changes in gene expression of cytokines were observed across different TRAP exposures in both females and males (Figure 7, Table S10). In males, a greater number of significant changes were observed in the lower respiratory airways than the upper airways. Anti-inflammatory IL-10 transcripts increased in the parenchyma by 1.8- to 3.8-fold (Figure 7A) and pro-inflammatory IL-18 transcripts increased in the distal airway by 1.17- to 1.25-fold in all TRAP exposures (Figure 7B) while these genes were largely unaffected or downregulated in the proximal airway and trachea (Figure 7C, D). In the upper airway trachea, there were no changes in any genes other than downregulation of IL-6 in PM(LDV). However, in the proximal bronchial airway, IL-1β was downregulated by 0.66- to 0.75-fold in all exposures (Figure 7C) and a similar response was observed in the alveoli-containing parenchyma (Figure 7A). In females, gene expression of IL-1β was downregulated in PM + Gas(LDV + HDV), PM(LDV + HDV), and PM(LDV) groups in the trachea (Figure 7D) and in Gas(LDV + HDV) and PM + Gas(LDV) groups in the proximal airway (Figure 7C). Yet, IL-1β was upregulated in all TRAP groups exposed to LDV + HDV emissions in the distal airway (Figure 7B). Additionally, tracheal IFN-γ gene expression was upregulated across most TRAP groups, whereas these changes were not observed in any other microdissected airway regions (Figure 7D).

Effects of TRAP on Inflammation and Recruitment of Immune Cells to the Lung across Different Post-Exposure Days

To compare the effects of TRAP on immune cell recruitment to the lung, H&E histology of the fixed lung and Hema-Diff

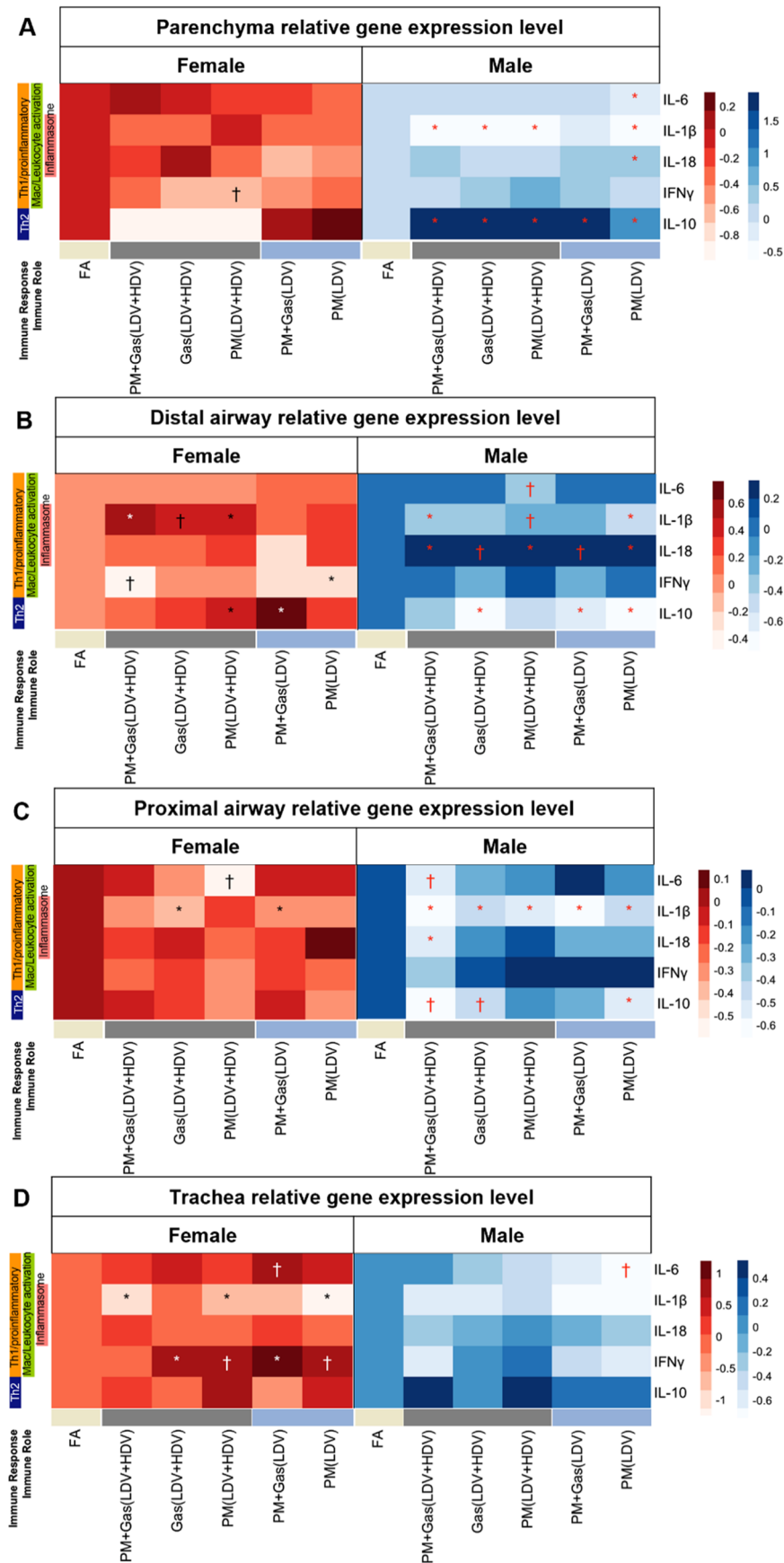


Figure 7. Sex-specific and lung region-dependent effects of TRAP on relative cytokine gene expression levels in 4 month-old TgF344-AD rats after 3 months of exposure in (A–C) airways microdissected from cranial lobe and (D) trachea. Data were analyzed using linear regression, followed by contrasts between TRAP groups and FA control with Dunnett’s adjustment for multiple comparisons; *p. adj < 0.05, †p. adj < 0.1. Female and

Figure 7. continued

male data are presented as a log₂ fold-change of exposure group mean normalized to sex- and age-matched FA control group mean. Sample size per group per sex ranged from $n = 4$ –7 across the cytokines after exclusion of statistical outliers.

histology of fixed BAL cells lavaged from the right lobes were assessed. H&E histology (Figure 8A) was semi-quantitatively scored based on (1) the volume of BALT formed with lymphocyte aggregates (Figure 8B) and (2) the presence of airway wall thickening (Figure 8C) as indications of leukocyte recruitment and inflammation. Overall, 3 months of exposure to TRAP did not cause prominent histopathologic inflammation or immune activation on the basis of these two criteria. However, PM + Gas(LDV + HDV), Gas(LDV + HDV), and PM(LDV) exposures moderately increased lymphocyte recruitment in the male lung, evident by a significantly higher proportion of elevated BALT scores compared to FA in the lower airway regions but not in the upper airway regions (Figure 8B, Table S11). Females exposed to TRAP had slightly higher BALT scores in the lower airway but not significantly and some significant increase in scores in the upper airway of PM(LDV). Only females exposed to Gas(LDV + HDV) exhibited a higher percentage of airway thickening observed compared to FA or any other groups including males, which did not show any obvious tissue thickenings (Figure 8C).

The BAL cells, which represent leukocyte populations in the airway and alveolar lumen, had no significant changes in the number of eosinophils, lymphocytes, or macrophages (Figure 9 and Table S12), but the number of neutrophils increased in response to TRAP exposure. While all 5 TRAP exposures increased the influx of neutrophils in the BAL in female animals, only groups that received the complete mix of PM + Gas had a statistically significant increase: 14.5-fold increase in PM + Gas(LDV + HDV) and 18.2-fold increase in PM + Gas(LDV) (Figure 9A). In males, neutrophil infiltration was only observed in groups that contained the PM component, PM + Gas(LDV + HDV), PM(LDV + HDV), and PM(LDV), yet the only statistically significant change was observed in PM(LDV) (Figure 9B).

Sex-Specific TRAP Effects on HMGB1 in the Serum and Lung Homogenate across Different Post-Exposure Days

To examine the effects of TRAP on HMGB1, which is a DAMP molecule released by macrophages and injured cells, an ELISA was used to measure the HMGB1 protein levels in the serum and lung tissue homogenates. Interestingly, while circulating HMGB1 in the serum was not impacted by TRAP exposure in females, it was significantly elevated in males across all TRAP exposure groups (Figure 10A). The magnitude of increase in serum HMGB1 was greater in males that were exposed to emissions from LDV+HDV, ranging between 2.8- and 3.4-fold increases, than those exposed to LDV emissions only, ranging between 1.9- and 2.0-fold increases (Table S13). However, HMGB1 levels in the lung homogenate were unaffected by TRAP in males but were significantly elevated in females exposed to PM + Gas(LDV + HDV) by 1.4-fold (Figure 10B, Table S13).

Correlational Analysis of Quantitative Measurements of Inflammation

To get a more comprehensive understanding of the dynamic modulation between the systemic and pulmonary inflammatory mediators, all quantitative inflammatory outcomes, except for histology scoring and relative gene expressions, were taken

together for pairwise correlational analysis (Figure S2A,B). In males, PM(LDV + HDV) had the most significant correlations between type 1 cytokines involved in acute inflammation and innate and adaptive leukocyte recruitment as well as a few correlations between Th2-polarizing cytokines within and across the plasma and lung (Table 2). Despite only a few significant correlations, PM(LDV) had particularly strong Th17-specific associations between plasma GRO and LIX ($r = 0.990$), as well as between plasma GM-CSF and BAL neutrophils ($r = 0.997$) (Table 2). Compared to other TRAP exposures, only PM(LDV) was strongly correlated with systemic GM-CSF and BAL neutrophils (Figure S2C). Because GM-CSF is involved in both neutrophil and macrophage chemotaxis, a subsequent analysis was performed and showed that plasma GM-CSF was only positively associated with BAL neutrophils and not with macrophages (Figure S2D). There were no significant correlations between any outcomes in PM + Gas(LDV + HDV) and PM + Gas(LDV), and only one significant association was observed between pulmonary fractalkine and IL-18 in Gas(LDV + HDV). Collectively, these associations indicate that PM from either LDV + HDV or LDV-only emissions most dynamically influenced cytokines involved in neutrophil chemotaxis and acute pulmonary and systemic inflammation along with regulatory type 2 adaptations in males.

In females, positive associations between cytokines were mostly observed with LDV + HDV exposures but not with LDV-only exposures (Figure S2B). Primarily, positive correlations between cytokines that promote type-1 inflammatory responses were observed. PM + Gas(LDV + HDV) induced correlational changes modulating systemic and pulmonary chemotaxis of monocytes, eosinophils, natural killer (NK) cells, and neutrophils (Table 3). PM(LDV + HDV) led to associations of chemokines of monocyte, macrophage, and neutrophil, as well as cytokines that can prolong chronic inflammation (Table 3). Distinct from these two groups, Gas(LDV + HDV) induced correlations between regulatory cytokines that promote Th2 polarization and cell proliferation in the lungs.

DISCUSSION

In this study, we demonstrated significant effects of TRAP PM vs. gases by sex derived from different vehicular sources on peripheral inflammatory mediators after 3 months of exposure to ambient levels of TRAP in TgF344-AD rats. Animals in this study experienced TRAP exposures that captured the timing, duration, concentration, and composition of ambient TRAP that humans experience in near-roadway environments. A recent publication from the same cohort of this study has shown that TRAP component-dependently accelerated the rate of amyloid plaque deposition in the brain of both male and female TgF344-AD rats,¹¹ which highlights the need to investigate the role of peripheral inflammation in mediating the TRAP effects on the brain. Additionally, we compared PM size distributions, mass concentrations, and number concentrations between LDV + HDV and LDV-only emissions to better characterize how distinct source- and component-

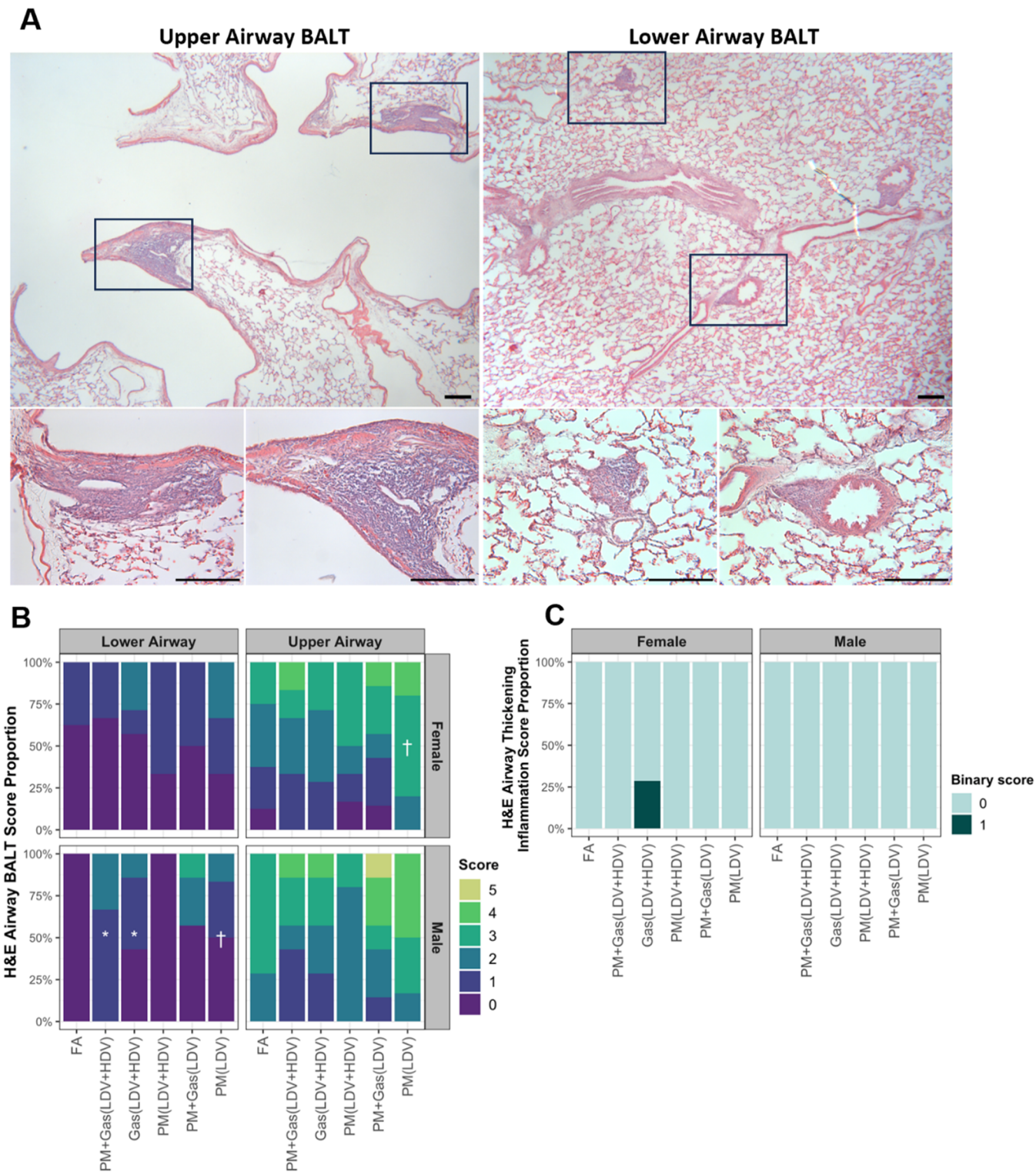


Figure 8. Sex- and lung region-dependent effects of TRAP on H&E histology in 4 month-old TgF344-AD rats after 3 months of exposure. (A) Example representative left lung lobe H&E photomicrographs of bronchus-associated lymphoid tissue (BALT) and airway wall thickening that were used as inflammatory indicators to semi-quantitatively score H&E images. Scale bar = 200 μ m; 4 $\times$ and 20 $\times$ magnifications. Semi-quantitative scoring of H&E histology data is presented as a group proportion of (B) BALT scores and (C) binary inflammation scores based on the volume of leukocyte aggregation and airway thickening, respectively. BALT score data (B) were analyzed using linear regression, followed by contrasts between TRAP groups and FA control with Dunnett's adjustment for multiple comparisons; **p*. adj < 0.05, †*p*. adj < 0.1. Sample size per group per sex ranged from *n* = 6–8 after exclusion of statistical outliers.

dependent biological responses may relate to differences in exposure profiles.

Our comparisons collectively showed that LDV + HDV emitted ultrafine PM, contributing negligible mass to the atmosphere due to their small size (<100 nm) but strikingly

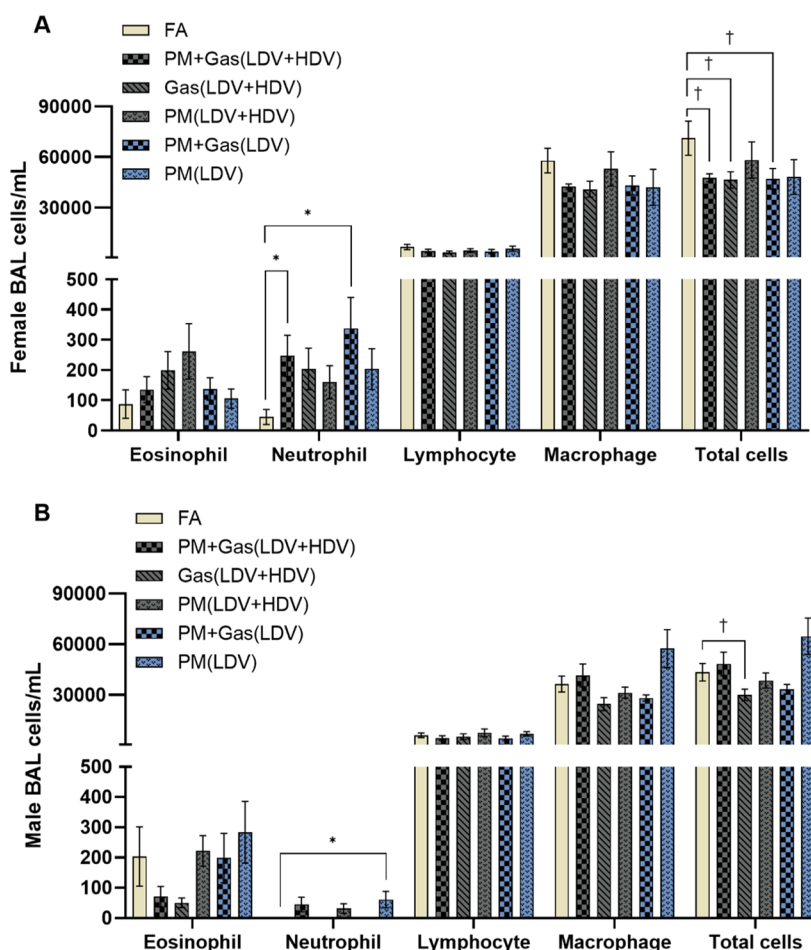


Figure 9. Sex-specific effects of TRAP on recruitment of immune cells in the BAL differentials from 4 month-old TgF344-AD rats after 3 months of exposure. Quantification of BAL eosinophils, neutrophils, lymphocytes, macrophages, and total cells in (A) females and (B) males. Data were analyzed using linear regression, followed by contrasts between TRAP groups and FA control with Dunnett's adjustment for multiple comparisons; **p*. adj < 0.05, †*p*. adj < 0.1. Data are presented as a group mean $\pm$ SEM (*n* = 6–9 per group per sex after exclusion of statistical outliers).

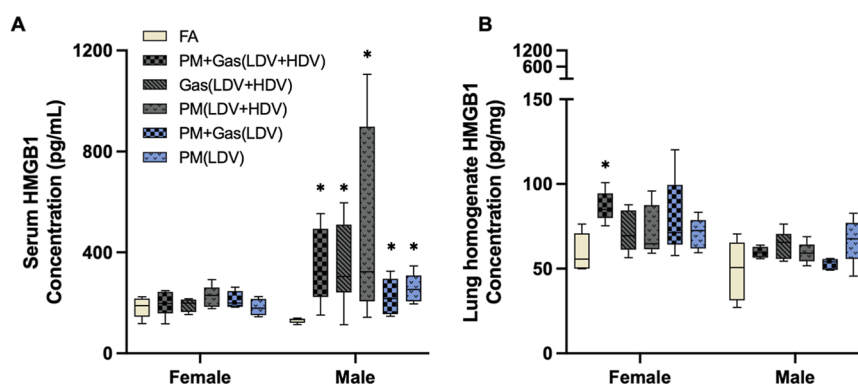


Figure 10. Sex-specific effects of TRAP on HMGB1 concentrations in (A) serum and (B) caudal lung homogenate in 4 month-old TgF344-AD rats after 3 months of exposure. Data were analyzed using linear regression, followed by contrasts between TRAP groups and FA control with Dunnett's adjustment for multiple comparisons; **p*. adj < 0.05, †*p*. adj < 0.1. Female and male data are presented as a group median with minimum and maximum values in a box and whiskers plot (*n* = 4–7 per group per sex after exclusion of statistical outliers).

higher number concentrations. Therefore, inter-bore differences in PM_1 – PM_{10} mass concentrations are negligible. In sharp contrast, vehicles emit very large numbers of ultrafine particles and emission rates are strongly dependent on vehicle type so that particle number concentration better traces differences in the vehicular fleet mixtures using each bore. Since HDV emission rates are an order of magnitude larger

than LDV emission rates,³³ the average number concentration in the LDV + HDV bore is significantly higher than the LDV-only bore. This has important implications for the study organ of interest considering growing evidence that pulmonary end points are generally more responsive to PM mass while neurological end points are more sensitive to particle size and number concentration.³⁴ Endotoxin levels in the PM samples

Table 2. Significant Correlations between Systemic and Respiratory Inflammation End Points in TRAP-Exposed Male TgF344-AD Rats

exposure group	end point 1	end point 2	Pearson's coefficient (r)	p. adj	biological processes	inflammation type
Gas(LDV + HDV)	Fractalkine_lung	IL-18_lung	0.997	0.027	chronic pulmonary inflammation with monocyte, T cell, and NK-cell recruitment	type 1
PM(LDV + HDV)	IFN γ _plasma	IL-17A_plasma	0.992	0.056	acute systemic inflammation with neutrophil and macrophage chemotaxis	type 1
PM(LDV + HDV)	IL-1 α _lung	IP-10_plasma	0.988	0.056	acute pulmonary inflammation with T-cell recruitment	type 1
PM(LDV + HDV)	IL-1 α _plasma	IL-12p70_plasma	0.988	0.056	systemic IFN γ production and Th1 polarization	type 1
PM(LDV + HDV)	IL-2_lung	EGF_plasma	0.987	0.056	pulmonary tissue remodeling and cell proliferation	type 2
PM(LDV + HDV)	IL-4_plasma	IL-18_plasma	0.987	0.056	systemic Th1/Th2 polarization feedback	type 1/2
PM(LDV + HDV)	GM-CSF_lung	IL-2_plasma	0.986	0.056	pulmonary myeloid activation and systemic T-cell activation	type 1
PM(LDV + HDV)	IL-18_lung	IP-10_plasma	0.986	0.056	pulmonary IFN γ production and systemic T-cell chemotaxis	type 1
PM(LDV + HDV)	G-CSF_plasma	IL-6_plasma	0.985	0.063	acute systemic inflammation with neutrophil chemotaxis	type 1
PM(LDV + HDV)	EGF_plasma	Eotaxin_plasma	0.997	0.015	systemic eosinophil chemotaxis and cell proliferation	type 2
PM(LDV + HDV)	IL-2_lung	Eotaxin_plasma	0.983	0.072	pulmonary T-cell activation and systemic eosinophilic chemotaxis	type 2
PM(LDV + HDV)	IL-18_lung	BAL neutrophil	−0.981	0.077	regulatory and resolution feedback of pulmonary neutrophil responses	type 1 with regulation
PM(LDV + HDV)	BAL macrophage	BAL total cells	0.983	0.004	normal pulmonary monocyte/macrophage response	homeostasis/type 1
PM(LDV)	GM-CSF_plasma	BAL neutrophil	0.997	0.015	systemic GM-CSF-induced increase in circulating neutrophils, activation/priming of neutrophils, intracellular calcium mobilization, and cytokine secretion	type 1, Th17
PM(LDV)	GRO_plasma	LIX_plasma	0.990	0.088	acute systemic inflammation with neutrophil chemotaxis	type 1, Th17
PM(LDV)	BAL macrophage	BAL total cells	0.992	0.0002	normal pulmonary monocyte/macrophage response	homeostasis/type 1

were measured to examine its potential contribution to inflammatory responses. The detected LPS level was approximately an order of magnitude lower than even the low end of what has been measured in other urban environments and several orders of magnitude lower than what is typically observed in rural air sheds.^{35–37} Thus, endotoxin is not considered a contributing factor to the outcomes reported here.

Total VOC (TVOC) concentrations at the CTEF were consistently below the detection limits (~50 ppb) of the various photoionization detectors deployed during these studies to measure isobutylene-equivalent TVOC, which includes all VOCs, intermediate VOCs (IVOCs), and semi-volatile VOCs (SVOCs) with ionization energies less than the 10.6 eV lamp used in following instruments: VOC-103L probe with ppb PID sensor (Graywolf Sensing Solutions; Shelton, CT), ppbRAE 3000, and ppbRAE 3000+ (Honeywell; Charlotte, NC, USA). This is due to a confluence of factors, but most importantly: (1) the rapidly increasing fraction of modern vehicle fleet mixtures comprising electric and hybrid vehicles, especially in the San Francisco Bay Area, CA, USA (Cheng and Lein, personal communication), and (2) the high VOC removal efficiencies of modern three-way catalytic converters (TWC) in LDVs and diesel oxidation catalysts (DOC) in HDVs, which are approximately 90–99% and 60–90% efficient, respectively, at steady-state operating conditions for TVOC in general, and alkenes, alkynes, aromatics, and oxygenated VOCs specifically.^{38–40} Furthermore, cold starts are not an issue at the CTEF since it is a freeway environment

with vehicles already operating at optimal temperatures where TWCs and DOCs are most efficient and the species that TWCs/DOCs are least efficient at removing, the low-molecular-weight saturated hydrocarbons, such as methane and ethane, are not ionized by the PID detectors. Therefore, it was not possible to determine inter-bore differences in TVOC emissions between the LDV + HDV and LDV-only bores and TVOC concentrations in general at CTEF are surprisingly low (<50 ppb isobutylene equivalents) so the toxicological relevance of TVOC compared to PM is unclear. Parallel arguments hold true for NO $_x$ as well given the high conversion efficiencies of TWCs in LDVs and selective catalytic reduction (SCR) employed in modern HDVs, especially in California, and inter-bore differences were unmeasurable using the electrochemical sensors deployed in these studies.

Since females and males were euthanized separately on nonoverlapping post-exposure days (Figure 1B), the effects of sex and post-exposure day were confounded and could not be independently evaluated. Therefore, our results and interpretations are limited to comparisons made between TRAP groups with sex-matched FA controls within each sex, rather than between sexes. We accounted for post-exposure day by including it as a covariate in the main effects linear regression model performed for all outcomes for each sex, yet this statistical adjustment may not fully detangle the confounding effect. Therefore, the findings reported here describe sex-specific effects of different TRAP components within each sex but may also be influenced by post-exposure day. An additional analysis that compares the effects of post-exposure days on

Table 3. Significant Correlations between Systemic and Respiratory Inflammation End Points in TRAP-Exposed Female TgF344-AD Rats

exposure group	end point 1	end point 2	Pearson's coefficient (r)	p. adj	biological processes	inflammation type
PM + Gas(LDV + HDV)	BAL macrophage	BAL total cells	0.980	0.007	normal pulmonary monocyte/macrophage response	homeostasis/type 1
PM + Gas(LDV + HDV)	IL-4_plasma	MIP-1 α _plasma	0.992	0.076	systemic eosinophil and monocyte chemotaxis	type 2
PM + Gas(LDV + HDV)	LIX_plasma	MIP-1 α _plasma	0.989	0.100	acute systemic neutrophil and monocyte recruitment	type 1
PM + Gas(LDV + HDV)	Eotaxin_plasma	IL-2_plasma	0.987	0.100	systemic T-cell activation and eosinophil chemotaxis	type 2
PM + Gas(LDV + HDV)	Fractalkine_lung	IP-10_lung	0.985	0.100	pulmonary monocyte, T cell, and NK-cell recruitment	type 1
PM + Gas(LDV + HDV)	IL-4_plasma	LIX_plasma	0.985	0.100	systemic neutrophil recruitment with tissue remodeling	type1/2
Gas(LDV + HDV)	BAL macrophage	BAL total cells	0.967	0.021	normal pulmonary monocyte/macrophage response	homeostasis/type 1
Gas(LDV + HDV)	IFN γ _lung	IL-13_lung	0.989	0.021	pulmonary Th1/Th2 polarization feedback	type 1/2
Gas(LDV + HDV)	EGF_lung	IL-18_lung	0.975	0.085	acute pulmonary inflammation with cell proliferation	type 1
Gas(LDV + HDV)	IFN γ _lung	IL-5_lung	−0.975	0.085	shifting to pulmonary Th2 polarization	type 1/2
PM(LDV + HDV)	BAL macrophage	BAL total cells	0.993	0.0001	normal pulmonary monocyte/macrophage response	homeostasis/type 1
PM(LDV + HDV)	IL-6_plasma	IL-12p70_plasma	0.996	0.024	acute systemic inflammation with Th1 polarization	type 1
PM(LDV + HDV)	G-CSF_lung	IL-18_lung	0.992	0.060	pulmonary neutrophil recruitment and IFN γ production	type 1
PM(LDV + HDV)	IFN γ _plasma	MIP-1 α _plasma	0.990	0.070	systemic macrophage activation and neutrophil recruitment	type 1
PM(LDV + HDV)	GM-CSF_lung	IL-10_lung	0.988	0.076	pulmonary myeloid activation with anti-inflammatory feedback	type 1 with regulation
PM(LDV + HDV)	GM-CSF_plasma	IFN γ _plasma	0.985	0.084	systemic monocyte/macrophage activation with TNF- α and IL-1 β production	type 1
PM(LDV + HDV)	Fractalkine_plasma	RANTES_plasma	0.984	0.084	chronic systemic inflammation with T cell and monocyte chemotaxis	type 1
PM(LDV + HDV)	IFN γ _plasma	LIX_plasma	0.984	0.084	acute systemic inflammation with neutrophil chemotaxis and monocyte/macrophage activation	type 1
PM(LDV + HDV)	IL-4_plasma	TNF α _plasma	0.983	0.085	acute inflammation with B-cell activation and macrophage priming	type 1/2
PM + Gas(LDV)	BAL macrophage	BAL total cells	0.988	0.001	normal pulmonary monocyte/macrophage response	homeostasis/type 1
PM(LDV)	BAL macrophage	BAL total cells	0.971	0.024	normal pulmonary monocyte/macrophage response	homeostasis/type 1

BAL neutrophil count within each sex (i.e., post-exposure day 1 vs 2 in females and post-exposure day 3 vs 4 in males) using the same main effects regression model showed no statistically significant differences between post-exposure days within each sex (Table S15). However, this may not adequately rule out the effects of post-exposure day on BAL neutrophil results.

Females and males not only showed distinct patterns of inflammatory responses to chronic exposure to TRAP but also exhibited different sensitivity to TRAP emission sources and components. Females responded to PM-containing exposures, specifically to PM(LDV + HDV), evidenced as systemic increases in cytokines that promote IFN- γ production and neutrophil and monocyte/macrophage chemokines. While these TRAP-induced changes in pro-inflammatory cytokines were not observed in the female lung itself, there was an increased influx of neutrophils in BAL collected from the female lung. In males, all TRAP exposures significantly elevated pulmonary-type 1 cytokines involved in pro-inflammatory pathways such as such chronic antigenic inflammatory responses, fever generation, Th1 cytokine

production, I κ B phosphorylation, and chemotaxis of NK cells and T cells. Interestingly, in contrast to females, the Gas(LDV + HDV) most prominently increased systemic type 1 and type 2 cytokines and chemokines in males. Collectively, females developed more severe type 1 systemic inflammation, while males developed more severe pulmonary type 1 inflammation but also exhibited more robust pulmonary and systemic type 2 adaptations. These adaptations in male respiratory inflammation may have mitigated the progression to systemic inflammation and containment of inflammation in the lung.

Our findings corroborate previously reported observations of increased systemic cytokines with air pollution exposures^{41–43} but also highlight novel findings on sex-specific responses to TRAP emission components and vehicular sources as evident in the responses in systemic and pulmonary cytokines. Additionally, we report different trends of changes in protein and gene expression of cytokines in the whole lung lobe, microdissected airways, and trachea, which contrast with results from other acute exposure studies.^{44–46} Notably, while protein levels of cytokines were minimally altered in female

lung after subchronic TRAP exposure, whole lung lobe gene expression analysis showed downregulation of IL-1 β and IL-18 but upregulation of IL-4 and IL-5 only in females across most TRAP groups but predominantly in the LDV+HDV exposure group. These findings indicate that pulmonary injury responses to TRAP subsided in female lungs but were sustained in male lungs. Although overt cell injury and necrosis or prominent inflammation was not observed by H&E histology, increased airway wall thickening, which can result from chronic inflammation and cell injury and potentially lead to fibrosis, was observed in females exposed to Gas(LDV+HDV). Upon closer regional examination of airways, IL-1 β transcripts were downregulated in the upper airway regions (trachea and proximal airway) but were upregulated in the distal airway in females with TRAP exposure. In males, TRAP decreased IL-1 β expression in the proximal airway but increased IL-18 expression in the distal airway, suggesting more active pro-inflammatory signaling in the upper airway than lower airway in both females and males. These airway level-dependent inflammatory patterns were also detected by H&E histology, which suggested a moderate increase in BALT in the lower, but not the upper, airway regions of male lungs exposed to PM + Gas(LDV + HDV) and Gas(LDV + HDV). Females exhibited a similar airway-dependent pattern, although the changes in BALT were milder and statistically insignificant. Unlike females, males had lower IL-1 β transcripts along with higher IL-10 expressions in the lung parenchyma across all TRAP exposures, which indicates ongoing resolution of parenchyma inflammation at the transcript level. These asynchronous and airway-dependent changes of cytokine gene expression and protein levels demonstrate heterogeneous impacts of air pollutants, which can have airway location-dependent deposition patterns, and compensatory down-regulation of pro-inflammatory cytokines to resolve respiratory inflammation and injury in response to subchronic ambient TRAP exposure.

We expected synergistic or additive effects of PM and gas components⁴⁷ on biological outcomes, but we observed distinct inflammatory profiles for each component. Exposure to PM or gas fractions of TRAP induced more robust biological responses than the combined exposure to PM + Gas. Moreover, although gases and different sizes of PM are known to reach and affect different airway regions,³² no consistent airway-dependent patterns of response to TRAP were observed across the different inflammatory readouts for each TRAP component. These observations may reflect: (1) altered aerodynamics in deposition, clearance, and dispersion of PM and gases in the airway when combined vs when administered in combination, (2) diffuse detection of signals from mixed biological responses to PM and gases combined especially when exposures occur chronically, and/or (3) different biological adaptations to combined vs. separate TRAP components after chronic exposure to very low, ambient levels of PM and gases. As stated, average PM_{2.5} concentration in TRAP fractions to which animals were exposed was only 10.8 $\mu\text{g}/\text{m}^3$, which is a factor of 3 lower than the US EPA National Ambient Air Quality Standards for 24 h PM_{2.5} (35 $\mu\text{g}/\text{m}^3$) but slightly higher than the annual standard (9 $\mu\text{g}/\text{m}^3$). It is not well understood whether gases change the toxicity of PM or how gases alone cause inflammation. These results warrant further investigation of the health effects of the gaseous mixture of NO_x, CO, and VOCs, which form a major part of TRAP, but are largely understudied.

Cytokines recruit and activate immune cells either locally or systemically initiate responses to tissue injury and to regulate inflammation via signaling by pathogen- or damage-associated molecular patterns (PAMPs/DAMPs).⁴⁸ Together, cytokines and HMGB1 have been shown to be important biomarkers of pulmonary inflammation in humans.^{49,50} HMGB1 is a type of DAMP molecule released into the extracellular space either passively by necrotic cells or actively by resident macrophages and injured cells.⁴⁸ When secreted, HMGB1 sequentially recruits and polarizes macrophages to produce more pro-inflammatory cytokines, which in turn promotes ROS signaling based on the HMGB1 redox state.^{51,52} We measured the total secreted extracellular level of HMGB1 in the serum and total pulmonary HMGB1 in the lung homogenates. The comparison between total systemic and pulmonary HMGB1 in the context of cytokine changes provides further information about the state of damage and inflammation following chronic exposure TRAP. Interestingly, we observed opposing trends in the effects of TRAP on systemic and pulmonary HMGB1 in female and male mice. Males had substantial increases in circulating HMGB1 but unchanged pulmonary HMGB1, across all TRAP exposures, whereas females had no changes in circulating HMGB1 but elevated pulmonary HMGB1 in the PM + Gas(LDV + HDV) group. These results imply that even after 3 months of exposure, TRAP persistently caused ongoing stress, necrosis, and damage affecting vascular permeability⁵³ in male lungs, causing HMGB1 release into the systemic circulation. This resulted in less retention of HMGB1 in the lungs to recruit more leukocytes, which is corroborated with increased pulmonary chemokines and cytokines. On the other hand, unchanged systemic HMGB1 suggests that females experienced less severe respiratory inflammation or quicker but inefficient resolution of respiratory inflammation that may have led to more severe systemic inflammation with an increase in systemic cytokines but retention of HMGB1 in the lung. These findings and interpretations are consistent with previous studies showing that males had higher levels of HMGB1 secretion into the circulation and corresponding toll-like receptor 4 (TLR4) expression, which were associated with exacerbated pulmonary arterial hypertension (PAH) progression, in rat models of PAH as well as in human endothelial and pulmonary artery smooth muscle cells.^{54,55} An *in vitro* study found that primary male mouse pulmonary endothelial cells were more prone to necrosis and release of HMGB1 compared to female pulmonary endothelial cells,⁵⁶ which also indicates increased vascular permeability in males. On the basis of these observations, we noted a difference in each sex of efficacy and timing for initiation and resolution of inflammation. While HMGB1 has been studied as an alarmin in the context of air pollution⁵⁷ as well as a potential systemic mediator that can alter glial expressions in the 5xFAD mouse model of AD,^{58,59} it has been mainly studied in males only and has not been compared across lung and serum *in vivo*.

To delineate the TRAP effects on each peripheral inflammatory outcome, we made direct comparisons between TRAP-exposed animals and FA controls for each outcome separately. However, given the complex modulatory interplay coordinated between inflammatory mediators,⁶⁰ we also examined correlations among all outcomes. Notably, the PM component alone most strongly influenced coordinated systemic and pulmonary inflammatory responses in males, although the type of response differed by vehicular sources (LDV + HDV vs LDV). These observations diverge from what

we observed in systemic inflammatory cytokines, which showed the most sensitive responses to Gas-only. Altogether, these separate and correlational analyses suggest selective susceptibility of systemic cytokine responses vs coordinated peripheral inflammation to Gas and PM, respectively. In females, an unexpected number of significant correlations was observed in FA controls (Table S14). Although the majority of correlations were involved in Th1/Th2 feedback regulation that are also observed in healthy individuals,⁶¹ a large number of correlations involving acute inflammation and leukocyte chemotaxis in FA controls is atypical. A potential explanation is that females may have been experiencing AD-related prodromal systemic inflammation, which has been implicated clinically in patients with mild cognitive impairment.⁶² Systemic inflammation plays a role in AD progression by contributing to BBB impairment and neuroinflammation,³ but these processes are interdependent with an unclear temporal sequence of events that may occur concurrently. Circulating amyloid β ($A\beta$), which can originate either from platelets and liver⁶³ or from brain clearance via cerebrospinal fluid, BBB transport, and glymphatic drainage,⁶⁴ can cause systemic inflammation via peripheral macrophages/monocytes activation.⁶⁵

Additionally, despite being exposed to clean air, FA controls still experienced acute stress as true controls for the study, such as transportation to the CTEF from UC Davis vivaria and traffic noise and vibration. These stressors can promote oxidative stress and inflammation via the HPA axis and stress hormone signaling,⁶⁶ which may have affected female FA controls due to underlying sex differences in stress hormonal responses.⁶⁷ Deviations from FA female correlations that we observed in TRAP groups, especially LDV-only groups that did not have any correlations between cytokines, may indicate TRAP-induced inflammatory dysregulation in addition to underlying AD-driven inflammation. Nevertheless, we cannot verify these speculations because wild-type rats and FA controls without stress were not included in the study. Directly comparing FA- and TRAP-exposed wild-type and transgenic rats could have delineated AD-specific and TRAP-specific dysregulation of peripheral inflammatory mediators. However, without wild-type controls, our findings and interpretations of TRAP effects are limited to the TgF344-AD rat model of AD, in which AD progression remains confounded. While the outcomes of the study did not include respiratory and neurological functional evaluations, previous studies have reported impaired contextual fear conditioning response in TgF344-AD rats¹⁰ and no significant genotype effects on respiratory control in the TgF344-AD rat model.⁶⁸

Although our data support TRAP-induced pulmonary inflammation and its progression to systemic inflammation, we cannot rule out other sources of inflammatory mediators since other peripheral organs chronically impacted by TRAP could have driven or contributed to systemic inflammation. Future studies would benefit the field by including an analysis of other organs. Despite efforts in the field, mechanisms underlying sex-dependent inflammatory responses and susceptibilities to inflammatory diseases and respiratory infections remain unclear. While roles of sex hormones, differential expression of TLRs, and X-chromosome-linked innate immune genes were suggested as potential explanations,^{69,70} there remains no clear consensus. Our observations on significant inflammatory responses by sex to different TRAP components raise questions that merit further exploration of underlying

component-specific sex differences in immune responses, regulation, and resolution after a chronic exposure of several months.

CONCLUSIONS

Our data collectively indicate that 3 months of ambient TRAP exposure induces sex-specific pulmonary and systemic inflammatory responses that vary by the TRAP component and emission source. Overall, TgF344-AD females developed type 1 systemic inflammation in response to PM(LDV + HDV), while TgF344-AD males developed pulmonary type 1 inflammation and exhibited stronger type 2 adaptations, largely driven by exposure to Gas (LDV + HDV). Across other end points, distinct inflammatory profiles were observed for each TRAP component in a sex- and airway region-specific manner. However, due to the confounding of sex and post-exposure day in this study, the observed sex-specific differences need to be further confirmed in follow-up studies that separate these two factors. Further analyses of chemical characteristics of PM and gas in our TRAP exposure are necessary to better understand which class of chemicals in TRAP-derived PM and gas drive the observed respiratory toxicity and how the combined exposure changes the aerodynamics of TRAP components and their biological effects in females and males. The effects of gas-phase components and PM from LDV emissions remain understudied. Therefore, studies that examine PM and gas, as well as the unchanged original source of TRAP, are important, especially over long time scales involving chronic exposures. Identifying such information can aid in developing more efficacious air filtration systems in automobiles and homes near roadways, promoting efficient and health-protective fuels and social engineering of communities, and implementing regulations and prevention to reduce negative health impacts of near-roadway TRAP.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/envhealth.6c00178>.

Additional gene ontology pathway analysis for elevated pulmonary cytokines, methods describing assay and qRT-PCR primer details, and statistical results of all outcomes (PDF)

AUTHOR INFORMATION

Corresponding Author

Laura S. Van Winkle — Center for Health and the Environment and Anatomy, Physiology and Cell Biology, Weil School of Veterinary Medicine, University of California, Davis, Davis, California 95616, United States; orcid.org/0000-0002-1689-4446; Email: lsvanwinkle@ucdavis.edu

Authors

Heui Hye Park — Molecular Biosciences, Weil School of Veterinary Medicine, University of California, Davis, Davis, California 95616, United States; orcid.org/0000-0002-5820-6226

Mei-Yun Cheng — Molecular Biosciences, Weil School of Veterinary Medicine, University of California, Davis, Davis, California 95616, United States

Anthony E. Valenzuela — Molecular Biosciences, Weil School of Veterinary Medicine, University of California, Davis, Davis, California 95616, United States

Morgan C. Domanico — Center for Health and the Environment, University of California, Davis, Davis, California 95616, United States

Christopher Wallis — Air Quality Research Center, University of California, Davis, Davis, California 95616, United States

Allyson Ikeda — Molecular Biosciences, Weil School of Veterinary Medicine, University of California, Davis, Davis, California 95616, United States

Lourdes Sanchez — Molecular Biosciences, Weil School of Veterinary Medicine, University of California, Davis, Davis, California 95616, United States

Allison Ehrlich — Department of Environmental Toxicology, University of California, Davis, Davis, California 95616, United States

Rachel Reader — California National Primate Research Center and Pathology, Microbiology and Immunology, Weil School of Veterinary Medicine, University of California, Davis, Davis, California 95616, United States

Vidya Chandrasekaran — Biology Department, Saint Mary's College of California, Moraga, California 94575, United States

Anthony S. Wexler — Air Quality Research Center and Mechanical and Aerospace Engineering, Civil and Environmental Engineering, and Land, Air and Water Resources, University of California, Davis, Davis, California 95616, United States; orcid.org/0000-0003-1565-814X

Keith J. Bein — Center for Health and the Environment and Air Quality Research Center, University of California, Davis, Davis, California 95616, United States; orcid.org/0000-0002-4865-2951

Pamela J. Lein — Molecular Biosciences, Weil School of Veterinary Medicine, University of California, Davis, Davis, California 95616, United States; Air Quality Research Center, University of California, Davis, Davis, California 95616, United States; orcid.org/0000-0001-7665-7584

Complete contact information is available at:

<https://pubs.acs.org/10.1021/envhealth.6c00178>

Author Contributions

H.H.P.: writing—original draft, conceptualization, investigation, formal analysis, software, visualization, project administration, and validation. M.Y.C.: investigation, project administration, and writing—review and editing. A.E.V.: project administration, investigation, supervision, and writing—review and editing. M.C.D.: investigation and writing—review and editing. C.W.: investigation, resources, supervision, and writing—review and editing. A.I.: investigation and writing—review and editing. L.S.: investigation and writing—review and editing. A.E.: investigation and writing—review and editing. R.R.: investigation and writing—review and editing. V.C.: project administration, resources, supervision, and writing—review and editing. A.S.W.: conceptualization, funding acquisition, resources, and writing—review and editing. K.J.B.: conceptualization, funding acquisition, resources, project administration, supervision, and writing—review and editing. P.J.L.: conceptualization, funding acquisition, resources, project administration, supervision, and writing—review and editing. L.S.V.W.: conceptualization, funding

acquisition, project administration, supervision, and writing—review and editing.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This research was supported by the National Institute on Aging (grant #RF1 AG074709 to P.J.L., L.S.V.W., and K.J.B.), the National Heart, Lung, and Blood Institute predoctoral training grant program (grant #T32 HL007013 to H.H.P. and M.C.D.), the National Institute of General Medical Sciences Maximizing Access to Research Career Undergraduate Student Training in Academic Research Award (grant #T34 GM13646 to L.S.), and the National Institute of Environmental Health Sciences center core grant (grant #P30 ES023513). The authors acknowledge Danielle Harvey from the Department of Public Health Sciences, UC Davis School of Medicine for her consultation on statistical analyses and Colin Reardon from Department of Anatomy, Physiology, and Cell Biology, UC Davis Weil School of Veterinary Medicine for granting access to QuantStudio6 Flex Real-Time PCR Systems. The authors would also like to express gratitude to the undergraduate students at Saint Mary's College of California who performed daily monitoring of animals during the exposure: Devin Billingsley, Kaylen Mohr, Casandra Lee, Roshan Thomas, Flona Gorgis, Joshua Galla, Sahiba Dogr. Figure ¹ was created with Biorender.

ABBREVIATIONS

AD	Alzheimer's disease
A β	amyloid β
AICc	Akaike Information Criterion (corrected)
ARRIVE	animal research: reporting of in vivo experiments
BBB	blood–brain barrier
BC	black carbon
BAL	bronchoalveolar lavage fluid
BALT	bronchus-associated lymphoid tissue
CTEF	Caldecott tunnel exposure facility
CO	carbon monoxide
DAM	damage-associated molecular patterns
DOC	diesel oxidation catalysts
FDR	false discovery rate
FA	filtered air
HDV	heavy-duty vehicle
H&E	hematoxylin and eosin
HMGB1	high mobility group box 1
IACUC	Institutional Animal Care and Use Committee
LDV	light-duty vehicle
LDV + HDV	light- and heavy-duty vehicle
LPS	lipopolysaccharide
NK cell	natural killer cell
NO _x	nitrogen oxide
O ₃	ozone
PAMP	pathogen-associated molecular patterns
PAH	pulmonary arterial hypertension
PAHs	polycyclic aromatic hydrocarbons
D _p	particle diameter
PM	particulate matter
PM + Gas	particulate matter and gas
PND	post-natal day
ROS	reactive oxygen species

SCR	selective catalytic reduction
SVOCs	semi-volatile organic compounds
SO ₂	sulfur dioxide
TLR	toll-like receptor
TSP	total suspended particle
TRAP	traffic-related air pollution
TWC	three-way catalytic converters
UC Davis	University of California, Davis
VOCs	volatile organic compounds.

REFERENCES

- (1) Fiordelisi, A.; Piscitelli, P.; Trimarco, B.; Coscioni, E.; Iaccarino, G.; Sorriento, D. The mechanisms of air pollution and particulate matter in cardiovascular diseases. *Heart Fail Rev.* **2017**, *22* (3), 337–347.
- (2) Wang, M.; Aaron, C. P.; Madrigano, J.; Hoffman, E. A.; Angelini, E.; Yang, J.; Laine, A.; Vetterli, T. M.; Kinney, P. L.; Sampson, P. D.; Sheppard, L. E.; Szpiro, A. A.; Adar, S. D.; Kirwa, K.; Smith, B.; Lederer, D. J.; Diez-Roux, A. V.; Vedral, S.; Kaufman, J. D.; Barr, R. G. Association Between Long-term Exposure to Ambient Air Pollution and Change in Quantitatively Assessed Emphysema and Lung Function. *JAMA* **2019**, *322* (6), 546–556.
- (3) Park, H. H.; Armstrong, M. J.; Gorin, F. A.; Lein, P. J. Air Pollution as an Environmental Risk Factor for Alzheimer's Disease and Related Dementias. *Med. Res. Arch* **2024**, *12*(10)..
- (4) Lao, X. Q.; Bo, Y.; Chen, D.; Zhang, K.; Szeto, C. C. Environmental pollution to kidney disease: an updated review of current knowledge and future directions. *Kidney Int.* **2024**, *106* (2), 214–225.
- (5) Fouladi, F.; Bailey, M. J.; Patterson, W. B.; Sioda, M.; Blakley, I. C.; Fodor, A. A.; Jones, R. B.; Chen, Z.; Kim, J. S.; Lurmann, F.; Martino, C.; Knight, R.; Gilliland, F. D.; Alderete, T. L. Air pollution exposure is associated with the gut microbiome as revealed by shotgun metagenomic sequencing. *Environ. Int.* **2020**, *138*, 105604.
- (6) Mortamais, M.; Gutierrez, L. A.; de Hoogh, K.; Chen, J.; Vienneau, D.; Carriere, I.; Letellier, N.; Helmer, C.; Gabelle, A.; Mura, T.; Sunyer, J.; Benmarhnia, T.; Jacquemin, B.; Berr, C. Long-term exposure to ambient air pollution and risk of dementia: Results of the prospective Three-City Study. *Environ. Int.* **2021**, *148*, 106376.
- (7) Iaccarino, L.; La Joie, R.; Lesman-Segev, O. H.; Lee, E.; Hanna, L.; Allen, I. E.; Hillner, B. E.; Siegel, B. A.; Whitmer, R. A.; Carrillo, M. C.; Gatsonis, C.; Rabinovici, G. D. Association Between Ambient Air Pollution and Amyloid Positron Emission Tomography Positivity in Older Adults With Cognitive Impairment. *JAMA Neurol* **2021**, *78* (2), 197–207.
- (8) Bendtsen, K. M.; Gren, L.; Malmberg, V. B.; Shukla, P. C.; Tuner, M.; Essig, Y. J.; Kraus, A. M.; Clausen, P. A.; Berthing, T.; Loeschner, K.; Jacobsen, N. R.; Wolff, H.; Pagels, J.; Vogel, U. B. Particle characterization and toxicity in C57BL/6 mice following instillation of five different diesel exhaust particles designed to differ in physicochemical properties. *Part. Fibre Toxicol.* **2020**, *17* (1), 38.
- (9) Herr, D.; Jew, K.; Wong, C.; Kennell, A.; Gelein, R.; Chalupa, D.; Raab, A.; Oberdorster, G.; Olschowa, J.; O'Banion, M. K.; Elder, A. Effects of concentrated ambient ultrafine particulate matter on hallmarks of Alzheimer's disease in the 3xTgAD mouse model. *Neurotoxicology* **2021**, *84*, 172–183.
- (10) Patten, K. T.; Valenzuela, A. E.; Wallis, C.; Berg, E. L.; Silverman, J. L.; Bein, K. J.; Wexler, A. S.; Lein, P. J. The Effects of Chronic Exposure to Ambient Traffic-Related Air Pollution on Alzheimer's Disease Phenotypes in Wildtype and Genetically Predisposed Male and Female Rats. *Environ. Health Perspect.* **2021**, *129* (5), 57005.
- (11) Nasim, N.; Cheng, M. Y.; Park, H. H.; Valenzuela, A.; Andrew, P.; Wexler, A. S.; Chandrasekaran, V.; Van Winkle, L. S.; Bein, K.; Lein, P. J. Comparing effects of gaseous vs. particulate components of traffic-related air pollution on Alzheimer's disease biomarkers in a genetically susceptible rat model. *Neurotoxicology* **2026**, *114*, 103472.
- (12) Mumaw, C. L.; Levesque, S.; McGraw, C.; Robertson, S.; Lucas, S.; Stafflinger, J. E.; Campen, M. J.; Hall, P.; Norenberg, J. P.; Anderson, T.; Lund, A. K.; McDonald, J. D.; Ottens, A. K.; Block, M. L. Microglial priming through the lung-brain axis: the role of air pollution-induced circulating factors. *FASEB J.* **2016**, *30* (5), 1880–1891.
- (13) Campagnolo, D.; Borghi, F.; Fanti, G.; Keller, M.; Rovelli, S.; Spinazzè, A.; Cattaneo, A.; Cavallo, D. M. Factors affecting in-vehicle exposure to traffic-related air pollutants: A review. *Atmos. Environ.* **2023**, *295*, 119560.
- (14) Lee, S. H.; Chen, Y. H.; Chien, C. C.; Yan, Y. H.; Chen, H. C.; Chuang, H. C.; Hsieh, H. I.; Cho, K. H.; Kuo, L. W.; Chou, C. C.; Chiu, M. J.; Tee, B. L.; Chen, T. F.; Cheng, T. J. Three month inhalation exposure to low-level PM_{2.5} induced brain toxicity in an Alzheimer's disease mouse model. *PLoS One* **2021**, *16* (8), No. e0254587.
- (15) Tolis, E. I.; Karanotas, T.; Svolakis, G.; Panaras, G.; Bartzis, J. G. Air quality in cabin environment of different passenger cars: effect of car usage, fuel type and ventilation/infiltration conditions. *Environ. Sci. Pollut. Res.* **2021**, *28* (37), 51232–51241.
- (16) Bein, K. J.; Wallis, C. D.; Silverman, J. L.; Lein, P. J.; Wexler, A. S. Emulating Near-Roadway Exposure to Traffic-Related Air Pollution via Real-Time Emissions from a Major Freeway Tunnel System. *Environ. Sci. Technol.* **2022**, *56*, 7083.
- (17) Szklarczyk, D.; Kirsch, R.; Koutrouli, M.; Nastou, K.; Mehryary, F.; Hachilif, R.; Gable, A. L.; Fang, T.; Doncheva, N. T.; Pyysalo, S.; Bork, P.; Jensen, L. J.; von Mering, C. The STRING database in 2023: protein-protein association networks and functional enrichment analyses for any sequenced genome of interest. *Nucleic Acids Res.* **2023**, *51* (D1), D638–D646.
- (18) Jiang, C.; Meng, L.; Zhu, W.; Shahzad, M.; Yang, X.; Lu, S. Housekeeping gene stability in pristane-induced arthritis and antigen-induced pulmonary inflammation of rats. *Inflamm. Res.* **2009**, *58* (9), 601–609.
- (19) Norman, O.; Koivunen, J.; Maki, J. M.; Pihlajaniemi, T.; Heikkinen, A. Identification of suitable reference genes for normalization of reverse transcription quantitative real-time PCR (RT-qPCR) in the fibrotic phase of the bleomycin mouse model of pulmonary fibrosis. *PLoS One* **2022**, *17* (10), No. e0276215.
- (20) Livak, K. J.; Schmittgen, T. D. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* **2001**, *25* (4), 402–408.
- (21) Mann, L. M.; Angus, S. A.; Doherty, C. J.; Dominelli, P. B. Evaluation of sex-based differences in airway size and the physiological implications. *Eur. J. Appl. Physiol.* **2021**, *121* (11), 2957–2966.
- (22) Klein, S. L.; Flanagan, K. L. Sex differences in immune responses. *Nat. Rev. Immunol.* **2016**, *16* (10), 626–638.
- (23) Aggarwal, N. T.; Mielke, M. M. Sex Differences in Alzheimer's Disease. *Neurol. Clin.* **2023**, *41* (2), 343–358.
- (24) Hurvich, C. M. T. C.-L.; Tsai, C. L. Regression and Time Series Model Selection in Small Samples. *Biometrika* **1989**, *76* (2), 297–307.
- (25) Chambers, J. M., Linear models. In *Statistical Models in S*, J. M., Chambers, T. J., Hastie, Eds.; Wadsworth & Brooks/Cole: 1992.
- (26) Tukey, J. W. *Exploratory Data Analysis*; Addison-Wesley, 1977.
- (27) Lenth, R. emmeans: Estimated Marginal Means, aka Least-Squares Means. <https://rvinth.github.io/emmeans/>.
- (28) Pustejovsky, J. E.; Tipton, E. Robust variance estimation with the clubSandwich package. <https://CRAN.R-project.org/package=clubSandwich>.
- (29) D'Evelyn, S. M.; Vogel, C.; Bein, K. J.; Lara, B.; Laing, E. A.; Abarca, R. A.; Zhang, Q.; Li, L.; Li, J.; Nguyen, T. B.; Pinkerton, K. E. Differential inflammatory potential of particulate matter (PM) size fractions from Imperial Valley, CA. *Atmos. Environ.* **2021**, *244*, 117992.
- (30) van Leenen, K.; Jouret, J.; Demeyer, P.; Vermeir, P.; Leenknicht, D.; Van Driessche, L.; De Cremer, L.; Masmeijer, C.; Boyen, F.; Deprez, P.; Cox, E.; Devriendt, B.; Pardon, B. Particulate matter and airborne endotoxin concentration in calf barns and their

association with lung consolidation, inflammation, and infection. *J. Dairy Sci.* **2021**, *104* (5), 5932–5947.

(31) Prunicki, M.; Cauwenberghs, N.; Ataam, J. A.; Movassagh, H.; Kim, J. B.; Kuznetsova, T.; Wu, J. C.; Maecker, H.; Haddad, F.; Nadeau, K. Immune biomarkers link air pollution exposure to blood pressure in adolescents. *Environ. Health* **2020**, *19* (1), 108.

(32) *Biological Disposition of Airborne Particles: Basic Principles and Application to Vehicular Emissions. Air Pollution, the Automobile, and Public Health*; National Academies Press (US); Watson AY, B. R., Kennedy, D., Eds.: Washington (DC), 1988.

(33) Lindhjem, C. E.; Pollack, A. K.; DenBleyker, A.; Shaw, S. L. Effects of improved spatial and temporal modeling of on-road vehicle emissions. *J. Air Waste Manage. Assoc.* **2012**, *62* (4), 471–484.

(34) Ishihara, Y.; Tanaka, M.; Nezu, N.; Ishihara, N.; Oguro, A.; Vogel, C. F. A. Pathways to the Brain: Impact of Fine Particulate Matter Components on the Central Nervous System. *Antioxidants* **2025**, *14* (6), 730.

(35) Hwang, S.; Park, D. U. Ambient Endotoxin and Chemical Pollutant (PM₁₀, PM_{2.5}, and O₃) Levels in South Korea. *Aerosol Air Qual. Res.* **2019**, *19* (4), 786.

(36) Morgenstern, V.; Carty, C. L.; Gehring, U.; Cyrys, J.; Bischof, W.; Heinrich, J. Lack of spatial variation of endotoxin in ambient particulate matter across a German metropolitan area. *Atmos. Environ.* **2005**, *39* (36), 6931–6941.

(37) Mueller-Anneling, L.; Avol, E.; Peters, J. M.; Thorne, P. S. Ambient endotoxin concentrations in PM₁₀ from Southern California. *Environ. Health Perspect.* **2004**, *112* (5), 583–588.

(38) Song, S.; Zhang, S.; Zhang, X.; Verma, P.; Wen, M. Advances in Catalytic Oxidation of Volatile Organic Compounds over Pd-Supported Catalysts: Recent Trends and Challenges. *Frontiers in Materials* **2020**, *7*, 595667.

(39) Tian, Z.; Ding, X.; Pan, H.; Xue, Q.; Chen, J.; He, C. A Review of Simultaneous Catalytic Removal of NO_x and VOCs: From Mechanism to Modification Strategy. *Catalysts* **2025**, *15* (12), 1114.

(40) Robles-Lorite, L.; Dorado-Vicente, R.; Torres-Jimenez, E.; Bombek, G.; Lesnik, L. Recent Advances in the Development of Automotive Catalytic Converters: A Systematic Review. *Energies* **2023**, *16* (18), 6425.

(41) Tablin, F.; den Hartigh, L. J.; Aung, H. H.; Lame, M. W.; Kleeman, M. J.; Ham, W.; Norris, J. W.; Pombo, M.; Wilson, D. W. Seasonal influences on CAPs exposures: differential responses in platelet activation, serum cytokines and xenobiotic gene expression. *Inhal Toxicol* **2012**, *24* (8), 506–517.

(42) Wilson, D. W.; Aung, H. H.; Lame, M. W.; Plummer, L.; Pinkerton, K. E.; Ham, W.; Kleeman, M.; Norris, J. W.; Tablin, F. Exposure of mice to concentrated ambient particulate matter results in platelet and systemic cytokine activation. *Inhal Toxicol* **2010**, *22* (4), 267–276.

(43) Pradhan, S. H.; Gibb, M.; Kramer, A. T.; Sayes, C. M. Peripheral (lung-to-brain) exposure to diesel particulate matter induces oxidative stress and increased markers for systemic inflammation. *Environ. Res.* **2023**, *231* (Pt 3), 116267.

(44) Kim, G. D.; Shin, D. U.; Song, H. J.; Lim, K. M.; Eom, J. E.; Lim, E. Y.; Kim, Y. I.; Song, J. H.; Kim, H. J.; Lee, S. Y.; Shin, H. S. Analysis of particulate matter-induced alteration of genes and related signaling pathways in the respiratory system. *Ecotoxicol. Environ. Saf.* **2024**, *281*, 116637.

(45) Yang, J.; Chen, Y.; Yu, Z.; Ding, H.; Ma, Z. The influence of PM_{2.5} on lung injury and cytokines in mice. *Exp. Ther. Med.* **2019**, *18* (4), 2503–2511.

(46) Fujii, T.; Hayashi, S.; Hogg, J. C.; Vincent, R.; Van Eeden, S. F. Particulate matter induces cytokine expression in human bronchial epithelial cells. *Am. J. Respir. Cell Mol. Biol.* **2001**, *25* (3), 265–271.

(47) Huang, Y. C.; Rappold, A. G.; Graff, D. W.; Ghio, A. J.; Devlin, R. B. Synergistic effects of exposure to concentrated ambient fine pollution particles and nitrogen dioxide in humans. *Inhal Toxicol* **2012**, *24* (12), 790–797.

(48) Bianchi, M. E.; Crippa, M. P.; Manfredi, A. A.; Mezzapelle, R.; Rovere Querini, P.; Venereau, E. High-mobility group box 1 protein

orchestrates responses to tissue damage via inflammation, innate and adaptive immunity, and tissue repair. *Immunol. Rev.* **2017**, *280* (1), 74–82.

(49) Andersson, U.; Ottestad, W.; Tracey, K. J. Extracellular HMGB1: a therapeutic target in severe pulmonary inflammation including COVID-19? *Mol. Med.* **2020**, *26* (1), 42.

(50) Chavda, V. P.; Bezbaruah, R.; Ahmed, N.; Alom, S.; Bhattacharjee, B.; Nalla, L. V.; Rynjah, D.; Gadanec, L. K.; Apostolopoulos, V. Proinflammatory Cytokines in Chronic Respiratory Diseases and Their Management. *Cells* **2025**, *14* (6), 400.

(51) Wang, J.; Li, R.; Peng, Z.; Hu, B.; Rao, X.; Li, J. HMGB1 participates in LPS-induced acute lung injury by activating the AIM2 inflammasome in macrophages and inducing polarization of M1 macrophages via TLR2, TLR4, and RAGE/NF- κ B signaling pathways. *Int. J. Mol. Med.* **2019**, *45* (1), 61–80.

(52) Salo, H.; Qu, H.; Mitsiou, D.; Aucott, H.; Han, J.; Zhang, X. M.; Aulin, C.; Erlandsson Harris, H. Disulfide and Fully Reduced HMGB1 Induce Different Macrophage Polarization and Migration Patterns. *Biomolecules* **2021**, *11* (6), 800.

(53) Shao, M.; Tang, S. T.; Liu, B.; Zhu, H. Q. Rac1 mediates HMGB1-induced hyperpermeability in pulmonary microvascular endothelial cells via MAPK signal transduction. *Mol. Med. Rep.* **2016**, *13* (1), 529–535.

(54) Zemskova, M.; McClain, N.; Niihori, M.; Varghese, M. V.; James, J.; Rafikov, R.; Rafikova, O. Necrosis-Released HMGB1 (High Mobility Group Box 1) in the Progressive Pulmonary Arterial Hypertension Associated With Male Sex. *Hypertension* **2020**, *76* (6), 1787–1799.

(55) Rafikov, R.; Nair, V.; Sinari, S.; Babu, H.; Sullivan, J. C.; Yuan, J. X.; Desai, A. A.; Rafikova, O. Gender Difference in Damage-Mediated Signaling Contributes to Pulmonary Arterial Hypertension. *Antioxid Redox Signal* **2019**, *31* (13), 917–932.

(56) Zemskova, M.; Kurdyukov, S.; James, J.; McClain, N.; Rafikov, R.; Rafikova, O. Sex-specific stress response and HMGB1 release in pulmonary endothelial cells. *PLoS One* **2020**, *15* (4), No. e0231267.

(57) Zou, W.; He, F.; Liu, S.; Pu, J.; Hu, J.; Sheng, Q.; Zhu, T.; Zhu, T.; Li, B.; Ran, P. PM_{2.5} Induced the Expression of Fibrogenic Mediators via HMGB1-RAGE Signaling in Human Airway Epithelial Cells. *Can. Respir. J.* **2018**, *2018*, 1–10.

(58) Ahmed, C.; Greve, H. J.; Garza-Lombo, C.; Malley, J. A.; Johnson, J. A., Jr.; Oblak, A. L.; Block, M. L. Peripheral HMGB1 is linked to O(3) pathology of disease-associated astrocytes and amyloid. *Alzheimers Dement* **2024**, *20* (5), 3551–3566.

(59) Greve, H. J.; Dunbar, A. L.; Lombo, C. G.; Ahmed, C.; Thang, M.; Messenger, E. J.; Mumaw, C. L.; Johnson, J. A.; Kodavanti, U. P.; Oblak, A. L.; Block, M. L. The bidirectional lung brain-axis of amyloid-beta pathology: ozone dysregulates the peri-plaque micro-environment. *Brain* **2023**, *146* (3), 991–1005.

(60) Lee, A. J.; Ashkar, A. A. The Dual Nature of Type I and Type II Interferons. *Front. Immunol.* **2018**, *9*, 2061.

(61) Ma, X.; Zhang, X.; Liu, J.; Liu, Y.; Zhao, C.; Cai, H.; Lei, W.; Ma, J.; Fan, H.; Zhou, J.; Liu, N.; Zhang, J.; Wang, Y.; Wang, W.; Zhan, P.; Zhang, X.; Zhang, Q.; Shi, K.; Liu, P. The correlations between Th1 and Th2 cytokines in human alveolar echinococcosis. *BMC Infect. Dis.* **2020**, *20* (1), 414.

(62) King, E.; O'Brien, J. T.; Donaghy, P.; Morris, C.; Barnett, N.; Olsen, K.; Martin-Ruiz, C.; Taylor, J. P.; Thomas, A. J. Peripheral inflammation in prodromal Alzheimer's and Lewy body dementias. *J. Neurol., Neurosurg. Psychiatry* **2018**, *89* (4), 339–345.

(63) Shi, M.; Chu, F.; Zhu, F.; Zhu, J. Peripheral blood amyloid-beta involved in the pathogenesis of Alzheimer's disease via impacting on peripheral innate immune cells. *J. Neuroinflammation* **2024**, *21* (1), 5.

(64) Casey, E.; Li, Z.; Liang, D.; Ebelt, S.; Levey, A. L.; Lah, J. J.; Wingo, T. S.; Huls, A. Association between Fine Particulate Matter Exposure and Cerebrospinal Fluid Biomarkers of Alzheimer's Disease among a Cognitively Healthy Population-Based Cohort. *Environ. Health Perspect.* **2024**, *132* (4), 47001.

(65) Vollmar, P.; Kullmann, J. S.; Thilo, B.; Claussen, M. C.; Rothhammer, V.; Jacobi, H.; Sellner, J.; Nessler, S.; Korn, T.;

Hemmer, B. Active immunization with amyloid-beta 1-42 impairs memory performance through TLR2/4-dependent activation of the innate immune system. *J. Immunol.* **2010**, *185* (10), 6338–6347.

(66) Kuntic, M.; Hahad, O.; Munzel, T.; Daiber, A. Crosstalk between Oxidative Stress and Inflammation Caused by Noise and Air Pollution-Implications for Neurodegenerative Diseases. *Antioxidants* **2024**, *13* (3), 266.

(67) Bangasser, D. A.; Wicks, B. Sex-specific mechanisms for responding to stress. *J. Neurosci. Res.* **2017**, *95* (1–2), 75–82.

(68) Lucking, E. F.; Murphy, K. H.; Burns, D. P.; Jaisimha, A. V.; Barry-Murphy, K. J.; Dhaliwal, P.; Boland, B.; Rae, M. G.; O'Halloran, K. D. No evidence in support of a prodromal respiratory control signature in the TgF344-AD rat model of Alzheimer's disease. *Respir. Physiol. Neurobiol.* **2019**, *265*, 55–67.

(69) Chamekh, M.; Deny, M.; Romano, M.; Lefevre, N.; Corazza, F.; Duchateau, J.; Casimir, G. Differential Susceptibility to Infectious Respiratory Diseases between Males and Females Linked to Sex-Specific Innate Immune Inflammatory Response. *Front. Immunol.* **2017**, *8*, 1806.

(70) Fuentes, N.; Silveyra, P. Endocrine regulation of lung disease and inflammation. *Exp Biol. Med.* **2018**, *243* (17–18), 1313–1322.