# Are Adverse Health Effects from Air Pollution Exposure Passed on from Mother to Child?
## Wildfire Exposure to Rhesus Monkeys

## Final Report

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## Abstract

The objectives of this study were to determine the long-term health impact of neonatal wildfire smoke exposure and assess whether adverse health effects from wildfire smoke exposure may be transmitted to offspring. To complete our objectives, we investigated a cohort of California National Primate Research Center (CNPRC) outdoor housed female rhesus monkeys that were born within three months prior to the Trinity and Humboldt County wildfires of June/July 2008. We conducted a nonterminal and minimally invasive study in eight-year-old adult female monkeys born in the spring of 2008 and exposed to wildfire smoke $PM_{2.5}$ as infants. As an experimental control for the effects of wildfire smoke $PM_{2.5}$, we compared our findings in eight-year-old adult female monkeys that were born in the spring of 2009 and not exposed to wildfire smoke $PM_{2.5}$ as infants. Our primary assessments for long-term health impacts of early life wildfire smoke $PM_{2.5}$ included (1) evaluation of the peripheral blood response to microbial ligands (n=30 per group) and (2) conduction of high-resolution computerized tomography (CT)(n=14 per group). We also determined if offspring from wildfire smoke $PM_{2.5}$-exposed female monkeys showed evidence of immune dysregulation relative to offspring from control female monkeys. When compared with control adult monkeys, peripheral blood cells from wildfire smoke $PM_{2.5}$-exposed adult monkeys showed altered cytokine synthesis when cultured with microbial ligands, as well evidence of lung remodeling in CT scans. Offspring of wildfire smoke $PM_{2.5}$-exposed adult monkeys displayed significant differences in immune profiles relative to offspring of control adult monkeys. We conclude that early life exposure to wildfire smoke $PM_{2.5}$ can result in immune and lung function decrements that persist with maturity and show evidence of multigenerational transmission of immune dysregulation as a result of maternal exposure.

## Executive Summary

Background

Wildfires are a significant source of air pollution and are predicted to increase in frequency as a result of climate change. Despite becoming a public health concern, the impact of acute wildfire smoke inhalation on development of chronic disease is unknown, particularly in susceptible populations such as young children. Inflammation of the respiratory tract is a consistent observation of acute air pollutant exposure in both children and adults. However, the establishment of persistent lung function decrements is a distinguishing characteristic of air pollutant exposures in children, with growing epidemiologic evidence to suggest health outcomes are retained with maturity and precede the development of chronic obstructive pulmonary disease in adults. Understanding the health effects of wildfire smoke exposure in pediatric populations is currently limited by restrictions on experimental assessment and invasive methodology. To address this knowledge gap in an animal model that is most relevant to the study of pediatric populations, we have previously reported evidence of immune dysregulation and lung function decrements in a cohort of adolescent rhesus macaque monkeys that were exposed as infants to high concentrations of ambient $PM_{2.5}$ from Northern California wildfires (Black, et. al. 2017). For this current study, we proposed to continue assessment of immune and pulmonary changes in adult rhesus macaque monkeys due to early life wildfire smoke exposure in adulthood. Because there is growing evidence of multigenerational effects from toxin exposures during early life, we also evaluated whether a maternal immune phenotype could be transmitted across generations by examining offspring of female wildfire smoke-exposed animals.

Methods

Adult female monkeys were born and reared in an outdoor environment within three months prior to the Trinity and Humboldt County summer wildfires in 2008, which produced significant episodes of $PM_{2.5}$ within 2.7 miles of the California National Primate Research Center (CNPRC). Offspring consisted of both male and female animals assessed between 1.10-2.72 years of age. Peripheral blood was collected from both adult monkeys and their offspring. High resolution computerized tomography (HRCT) imaging of the thoracic cavity was conducted on adult animals only. Peripheral blood mononuclear cells (PBMC) were cultured with lipopolysaccharide (LPS) and assessed by Luminex and ELISA. CT scans were analyzed using Functional Respiratory Imaging technology to quantify structural changes and functional deficits. Because rhesus monkeys are seasonal breeders, a second cohort born in the spring of 2009 served as a control group; biospecimen collection and imaging were conducted in subsequent years to normalize for age.

Results

At eight years of age, adult female monkeys born in 2008 weighed significantly less than age-matched animals born in 2009. There were no differences in weight of female offspring from adult female monkeys evaluated in this study, however male offspring from female monkeys born in 2008 weighed significantly less than male offspring from female monkeys born in 2009. Following LPS treatment, PBMC cultures generated from peripheral blood samples of adult female monkeys exposed to early life wildfire smoke $PM_{2.5}$ showed increased expression of proinflammatory cytokines (interleukin-6, interleukin-1β, macrophage inflammatory protein-1β) and chemokine ligand 18 relative to controls. Independent of LPS treatment, levels of monocyte chemoattractant protein-1 and macrophage derived chemokine protein in PBMC cultures were reduced in adult female monkeys exposed to early life wildfire smoke $PM_{2.5}$. Serum C-reactive protein was also reduced in peripheral blood from exposed adult female monkeys. Offspring of female adult monkeys exposed to early life wildfire smoke $PM_{2.5}$ displayed significantly reduced expression of the proinflammatory cytokine interleukin-8, both in serum and in PBMC cultures following LPS treatment. HRCT scans from adult female monkeys exposed to early life wildfire smoke $PM_{2.5}$ showed reductions in lung volume, inspiratory capacity, and ventilation perfusion relative to adult female monkey controls. An increase in specific airway radius and blood vessel density was also detected in CT scans from exposed adult monkeys relative to control adult monkeys

Conclusions

Our findings demonstrate that early life ambient wildfire smoke $PM_{2.5}$ exposure is associated with dysregulation of immune responses, lung function decrements and airways remodeling that persists into adulthood. We further provide evidence of multigenerational transmission of an altered immune phenotype as a result of maternal wildfire smoke exposure. The observation of significant compromise of immune function in association with lung function decrements in adult female rhesus monkeys exposed to early life wildfire smoke $PM_{2.5}$ suggests that young human subjects who were similarly exposed to wildfire smoke $PM_{2.5}$ in 2008 could exhibit a similar health profile, with the caveat that the animals evaluated in this study were housed outdoors throughout the 2008 wildfire event.

## Introduction

As climate change continues and housing density near rural regions increases, wildfires are predicted to escalate in severity, frequency, and proximity to populated areas (1, 2). Wildfire events pose a direct health hazard to human inhabitants of affected areas due to burn injuries and exposure to toxic fumes from housing materials. Wildfires also elicit a significant amount of fine particulate matter into the atmosphere that can travel hundreds of miles from the site of origin, but there is currently little known about the public health outcomes of smoke exposures outside the immediate perimeter of burned regions (3, 4). Exposure to ambient fine particulate matter (aerodynamic diameter < 2.5 μm; $PM_{2.5}$) has been reported to elicit adverse effects on human health, with direct links to cardiovascular and pulmonary emergencies. There is also growing evidence that not all $PM_{2.5}$ are equivalent with regards to toxicity; health outcomes can differ widely depending upon geographic locations of $PM_{2.5}$ (5). Data on the impact of chemical composition for wildfire derived $PM_{2.5}$ on human health are currently limited, however, it has been reported in murine studies that collected wildfire $PM_{2.5}$ is more toxic on an equal mass basis than $PM_{2.5}$ derived from other combustion-sources (6, 7).

Experimental studies linking air pollutant exposures with pathologic outcomes in the respiratory tract has been well-documented in multiple laboratory animal models (reviewed in (8, 9)). For human subjects, direct evidence for a causal relationship between air pollution exposure and respiratory disease is controversial due to statistically confounding variables imposed by geographic locations, diet, and other environmental factors. A recent retrospective analysis of individuals born during the 1952 Great Smog in England showed increased frequency of self-reported childhood asthma in association with a 4 day window of high $PM_{10}$ concentrations, a finding notable for showing linkage of disease with a limited period of extreme exposure (10). Multiple physiologic parameters are believed to enhance susceptibility to air pollution health effects in children, such as increased metabolic rate and larger lung surface area per unit of body weight compared to adults (11).

Health effects of $PM_{2.5}$ and ozone in children have been given special consideration by the Environmental Protection Agency and guidelines have been established based upon epidemiologic studies linking reduced lung function with ambient air pollution exposure in children (12-15). Exposure to high levels of $PM_{2.5}$ during early life has been associated with long-term changes in pulmonary function and immunity (16-18). An observed increased respiratory tract infection for pediatric populations in association with elevated particulate matter has also been reported, which suggest a link between air pollutant exposures and host pathogen defense deficits in young children (19-21). Early life exposure to $PM_{2.5}$ has been associated with increased prevalence of asthma, allergies, and upper respiratory infections in young children (22, 23), as well as decreased force expiratory volume in one second ($FEV_1$) and forced vital capacity (FCV) later in life (17, 18, 24). Studies have also shown that exposures outside of the first year

life had no association with lung function decrements later in life (18, 25), reinforcing the notion of a critical window of susceptibility to air pollution during infancy.

Environmental exposures may lead to multigenerational effects, which are heritable phenotypic changes that are passed through male and female germlines to offspring (26). Although the exact mechanisms of transmission are still unclear, many animal studies have suggested that these effects are mediated through heritable epigenetic changes in DNA methylation, histone modification, and noncoding RNA expression (reviewed in (27)). Rodent studies have been conducted to experimentally demonstrate evidence of both multigenerational and transgenerational effects from chronic tobacco smoke exposure (28), however to the best of our knowledge, no other study has investigated the potential for $PM_{2.5}$ or wildfire smoke to elicit multigenerational or transgenerational effects.

The research proposed in this study is focused on determining whether an early life acute $PM_{2.5}$ exposure event can elicit health outcomes that persist into adulthood and may be transmitted to offspring. We have previously reported that air pollutant exposure to infant rhesus monkeys significantly alters innate immune responses later in life (25, 29). However, the progression of immune and respiratory changes as a result of early life acute $PM_{2.5}$ exposure events and relation to disease is currently unknown. The scope of the work described in this report exclusively utilizes non-invasive measures of health outcomes following early life exposure to wildfire smoke derived $PM_{2.5}$, such that long term longitudinal assessment and potential translation of findings to human subjects is feasible.

In this study, we evaluated age-matched cohorts of California National Primate Research Center (CNPRC) adult female rhesus macaque monkeys that experienced significantly different levels of wildfire derived $PM_{2.5}$ during infancy. Outdoor-housed rhesus monkeys born in the spring of 2008 were exposed to high levels of $PM_{2.5}$ derived from the Trinity/Humboldt wildfire episodes in June and July of that year. Outdoor-housed rhesus monkeys born in the spring of 2009 experienced normal levels of urban-based $PM_{2.5}$. Adult animals were non-invasively evaluated for immune and respiratory function by analysis of peripheral blood samples and imaging of the thorax. Additionally, offspring from females were also studied by evaluation of peripheral blood samples and cell culture-based analysis. Based upon the current population of animals at the CNPRC, there were insufficient numbers of exposed males born in 2008 available to provide offspring for assessment of paternal line effects in this study.

## Materials and Methods

Air Quality Data

During the summer of 2008, the Sacramento valley experienced multiple days of elevated concentrations of particulate matter, due to persistent ambient wildfire smoke from Northern California fires. As shown in Figure 1, a comparison of daily 8-hour ozone concentration detected by a California Air Resources Board sampling station in Yolo County located within 2 miles of the CNPRC shows similar patterns between June/July 2008 versus June/July 2009, with a single day in June 2008 and a single day in July 2008 that exceeded the current NAAQS standard of 0.075 ppm/8-hour period. In comparison, there were two episodes in the months of June and July 2008, consisting of 4-6 days each, where $PM_{2.5}$ levels exceeded the current NAAQS standard of 35 ug/$m^3$ per 24-hour period (Figure 2). $PM_{2.5}$ levels correlated with a dry low-pressure system on June 20-22, 2008 that produced dry lightning igniting approximately 2000 forest fires across Humboldt County in Northern California, which is located approximately 270 miles from the University of California, Davis campus. In Yolo County, where the CNPRC is located, air quality improved June 26-July 5 2008 due to onshore winds and Delta breeze, but declined July 7-10 2008 when winds calmed. In addition to the data presented within the time frame from Figures 1 and 2, the 8-hour daily average for ozone concentration was 0.099 ppm in 2008 and 0.082 ppm in 2009. Although the 8-hour daily average ozone declined between 2008 and 2009, it should be recognized that these values still exceed the state standard of 0.070 ppm. While these data represent the most accurate measures of air quality within the immediate vicinity of the CNPRC, it should be acknowledged that other sources of inhaled materials may have been present during the period of time in which infant monkeys were housed outdoors. For example, properties adjacent to the CNPRC consist of University of California, Davis agricultural fields; pesticide use is restricted but plant-derived allergens are unavoidable.

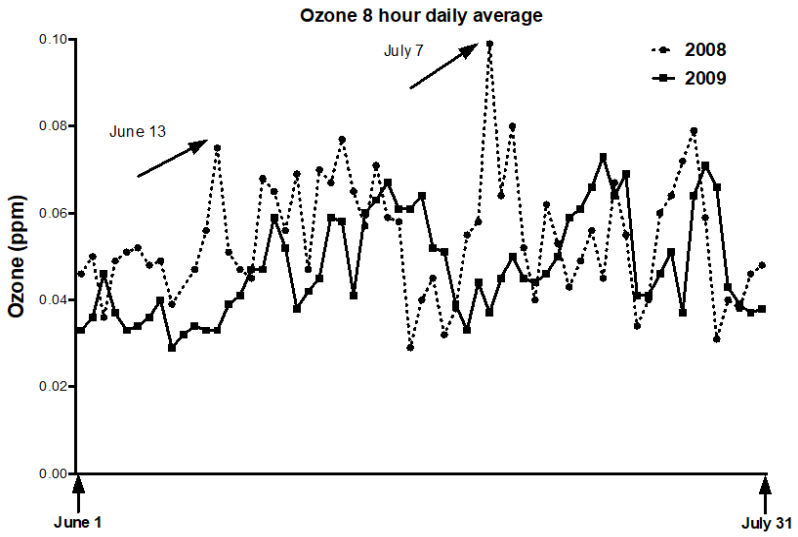


**Figure 1. Daily 8 hour average concentration of ozone from June 1-July 31 on UC Davis campus.** Daily average readings for ozone were obtained from a California Air Resources Board sampling site located on the UC Davis campus, within two miles of the California National Primate Center. Daily readings for 2008 (dotted line) and 2009 (solid line) are compared over time, arrows point to two different time points that exceed the current NAAQS standard of 0.075 ppm/8 hours. Note that there are 1-3 days in June with missed readings.

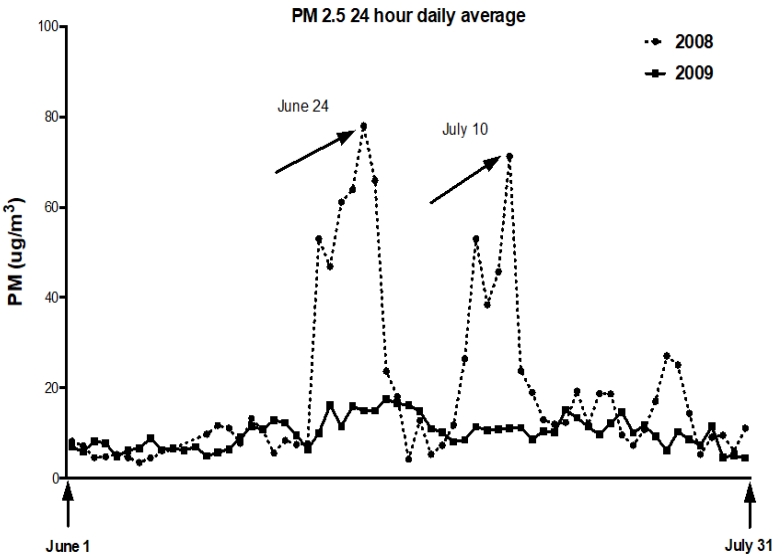


**Figure 2. Daily 24-hour average concentration of $PM_{-2.5}$ from June 1-July 31 on UC Davis campus.** Daily average readings for $PM_{2.5}$ were obtained from a California Air Resources Board sampling site located on the UC Davis campus, within two miles of the CNPRC. Daily readings for 2008 (dotted line) and 2009 (solid line) are compared over time, arrows point to the peak of two different time periods that exceed the current NAAQS standard 35 ug/$m^3$ per 24 hour period.

Animals

To determine the potential long-term health impacts of acute $PM_{2.5}$ exposures, we evaluated parameters of immune and respiratory function in outdoor housed CNPRC rhesus monkeys. Genetically diverse, eight-year-old adult female rhesus macaque monkeys (*Macaca mulatta*) were born and raised in CNPRC outdoor housing within three months prior to the Trinity and Humboldt County summer wildfires in 2008. Adult female rhesus macaque monkeys born and raised in CNPRC outdoor housing in 2009 served as exposure controls. Enrolled study animals were exclusively housed outdoors prior to the assessment period. Study animals were selected from more than 15 different CNPRC outdoor field cages that house multiple genetically diverse social groups. CNPRC social groups in outdoor housing may consist of familial lines or newly introduced animals, with an age range spanning from birth through geriatric. The CNPRC electronic medical record system was used to initially identify potential subjects based upon sex and birth date relative to the peak of $PM_{2.5}$ in June/July (approximately three months of age); study animal cohorts were established by random selection of females from the pool of identified potential subjects. Control animals were comparably selected (born within identical seasonal time frame) and age-matched to exposed animals. Offspring for the study were identified by known pedigree to study female monkeys (determined by DNA analysis conducted by UC Davis Veterinary Genetics Laboratory), sex and age. The age range of offspring was 1.1-2.72 years of age. Both male and female offspring were enrolled as study animals. A schematic of the study design is shown is Figure 3.

CNPRC animals housed in outdoor field cages are fed a commercial diet (Lab Diet #5038, Purina Mills International, St. Louis, MO) supplemented with fruits and vegetables. CNPRC produce is seasonal and sourced from a single vendor. Dams nurse infant monkeys until approximately 3 months of age. Developmental stages in the rhesus monkey are as follows: newborn, 24 hours postnatal; neonate, 0–1 months; infant, 1–12 months; juvenile, 12–24 months; adolescent, 2–4 years; and young adult, 4–8 years. Peripheral blood for serum and PBMC isolation was collected from adult animals and their offspring in outdoor field cages during bi-annual comprehensive health assessments conducted by CNPRC veterinary staff. Thoracic imaging was separately conducted on animals that were transported from outdoor field cages. Because rhesus monkeys are seasonal breeders, a second cohort of study animals born in the spring of 2009 served as the control group; biospecimen collection and imaging were staggered in subsequent years such that age at evaluation was held constant. Offspring of the adult control group served as the age-matched control of offspring from the adult wildfire smoke $PM_{2.5}$ group. Care and housing of animals before, during and after evaluations complied with the provisions of the Institute of Laboratory Animal Resources and conforms to practices established by the American Association for Accreditation of Laboratory Animal Care (AAALAC). The University of California Institutional Animal Care and Use Committee approved all animal procedures.

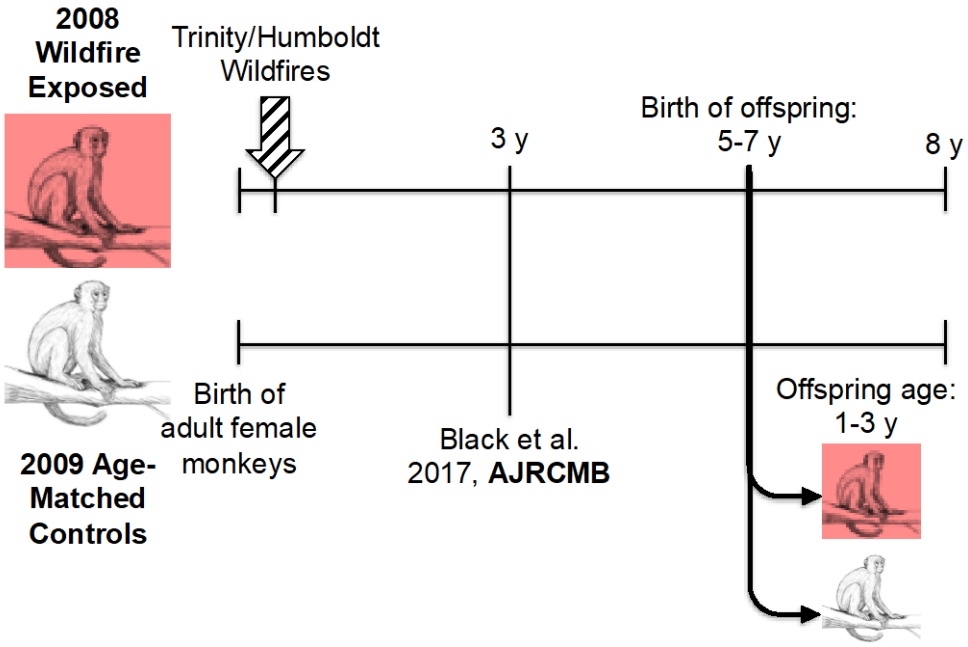


**Figure 3. Schematic of exposure and assessment timeline for study adult female monkeys and offspring.** As described in Materials and Methods, adult female monkeys were assessed at eight years of age for this study. Offspring of adult female monkeys were assessed at between one to three years of age. We have previously reported findings of wildfire smoke exposure on CNPRC animals assessed at three years of age (Black, et. al. 2017).

*In Vitro* Stimulation of Peripheral Blood Mononuclear Cells with Microbial Ligands

To assess the responsiveness of innate immune function in animals evaluated in this study, we tested cultures of peripheral blood cells by *in vitro* stimulation with microbial ligands. Peripheral blood was evaluated from 30 animals per study group (Adult females born in 2008 n=30; Offspring from females born in 2008 n=30; Adult females born in 2009 n=30; Offspring from females born in 2009 n=30). Peripheral blood mononuclear cells (PBMC) were used in the study as this population consisting of lymphocytes and monocytes is known to express Toll-like receptors that recognize a broad range of microbial ligands. Peripheral blood was evaluated from 30 study animals per study group (Adult females born in 2008 n=30; Offspring from females born in 2008 n=30; Adult females born in 2009 n=30; Offspring from females born in 2009 n=30). PBMC were prepared from blood samples by Histopaque 1077 gradient centrifugation and cryopreserved prior to culture (30). For consistency, experiments with thawed peripheral blood mononuclear cells were conducted after all blood samples were collected from a single year. Cryopreserved PBMC were revived and allowed to equilibrate for 1 h at 37°C. Thawed peripheral blood mononuclear cells were cultured in serum free AIM-V medium supplemented with 2 mM L-Glutamine (Invitrogen, Carlsbad, CA) in 96 well tissue culture plates at a concentration of 2 x $10^5$ cells/100 ml. Lipopolysaccharide (LPS)(E. Coli 026:B6, Sigma-Aldrich, St. Louis, MO) was diluted in AIM-V media at a concentration of either 5 or 50 ng/ml and added to cultures at the start of incubation. Cultures were maintained for 6 or 24 hours at 37°C in 5% $CO_2$. At the end of 6 hours (ELISA) or 24 hours (Luminex), media supernatants were collected by centrifugation of cultures to remove cells. Supernatant was stored at -80°C until analysis by ELISA or Luminex. All assays were conducted with identical media and reagent lots.

ELISA/Luminex Assays

IL-6 and IL-8 protein concentration in serum and PBMC culture supernatants were measured by ELISA. IL-6 ELISAs were performed with human IL-6 ELISA kit (Invitrogen, San Diego, CA). IL-8 ELISAs were performed with the human IL-8 DuoSet ELISA kit (R&D Systems, Minneapolis, MN). Rhesus C-reactive protein (CRP) ELISA was performed with the Monkey CRP ELISA kit (Life Diagnostics, West Chester, PA). Rhesus anti-Tetanus Toxoid ELISAs were performed with the human Tetanus IgG ELISA kit (IBL, Minneapolis, MN). Luminex was performed using the Monkey Cytokine 29-plex panel (Thermo Fisher Scientific, San Diego, CA). All ELISA/Luminex samples were conducted in duplicate. ELISA was conducted for all study animals. Luminex was conducted on a subset of study adult female monkeys (n=14 adult female monkeys exposed to early wildfire smoke $PM_{2.5}$; n=14 adult female monkey controls).

High Resolution Computed Tomography Imaging

Thoracic imaging was conducted to obtain non-invasive measures of lung function and remodeling in animals following acute $PM_{2.5}$ exposures. Imaging data was obtained from 14 animals per study group (Adult females born in 2008 n=14; Adult females born in 2009 n=14). Adult female monkeys used for high resolution computed tomography (HRCT) imaging studies were inspected via ultrasound to ensure less than 30 days of gestation prior to imaging procedures. Animals were sedated and intubated for HRCT imaging. HRCT images were collected on a GE Discovery 610 PET/CT Imaging System. HRCT

images were performed at 3 separate external pressures: 20 mm Hg (full), 10 mm Hg (partial), and 0 mm Hg (baseline).

Functional Respiratory Imaging Analysis

To quantify physiologic and structural changes in the lung of animals undergoing HRCT, scan data was converted into 3D models of airways and lung lobes using Mimics (Materialise, Leuven, Belgium) a commercially available validated software package (Food and Drug Administration, K073468; Conformité Européenne certificate, BE 05/1191.CE.01). Other software used included; TGrid 14.0 (Ansys Inc, Canonsburg, PA) for 3D meshing and Fluent 14.0 (Ansys Inc, Canonsburg, PA) for CFD simulations.

Segmentation of the tracheobronchial tree was done using directional thresholding with automated leakage detection. Automatic airway segmentation was performed up to the point where no distinction could be made between the intra-luminal and alveolar air. Following automated segmentation of the bronchial tree, the airways were manually checked. Missing branches were added to the bronchial tree and incorrect branches were deleted when necessary. The respiratory tract was reconstructed down to the level of airways with a diameter of 1–2 mm, beyond this point, the HRCT resolution is insufficient to distinguish alveolar from intraluminal air. The segmented airway tree was converted into a 3D model that was smoothed using a volume compensation algorithm. The smoothed model was trimmed perpendicular to the airway centerline at the trachea (using the middle point of the superior side of the sternum as a landmark) and at each terminal bronchus. Remaining artifacts due to noise in the HRCTs were then manually removed from the model. Finally, a series of manual quality checks were performed. Total time for the automated steps and manual quality checks varied from 2 to 6 hours per scan.

Functional Respiratory Imaging (FRI) is comprised of a combination of airway segmentation, lung volume segmentation by lobe, and airway resistance calculations based on computational flow simulation using boundary conditions provided by the lobe expansions from FRC to TLC, allowing calculation of lobe volume (iVlobe), airway volume (iVaw), as well as their specific values (corrected for lobe volume). Values for these parameters were calculated on a lobar level. By means of application of CFD on the segmented airway model, the airway resistance (iRaw) was calculated (31). Lungs were split into lobes by identification of the fissure lines from the CT scan. This allowed determination of total lung volume and of the volume of each lobe individually.

FRI has been validated by comparison with gamma scintigraphy and single-photon emission computerized tomography (31, 32).

Statistical Analysis

Statistical analysis was performed using Graphpad Prism 8.0 software (San Diego, CA). Significance between adult groups or offspring groups was determined with one-way ANOVA or Welch's t-test. All data are reported as mean +/- SD or mean +/- SEM as appropriate for each measured parameter. A p <value of 0.05 or less was considered statistically significant.

## Results

Summary of Animals Evaluated

As shown in Table 1, adult female monkeys surveyed within this study were identified based upon age relative to the peak $PM_{2.5}$ exposure period in 2008. Offspring from female monkeys born in 2008 were identified based upon pedigree analysis (Table 2). Age-matched female monkeys born in 2009 and their respective offspring were selected in an analogous fashion (Table 3). To compare outcomes from our previously reported study (33), we obtained blood samples from animals during field cage health assessments that take place in the months of June-November.

There were no significant differences in age of animals relative to June 24 2008 (2008 births or June 24 2009 (2009 births), a date that was selected as peak of $PM_{2.5}$ exposure in 2008. There were significant differences in weight between 2008 and 2009 females at the time of blood collection (2008: 4.868 kg $\pm$ 0.5847 SD; 2009: 4.589 kg $\pm$ 0.4669 SD). Linear regression analysis of body weight from birth year through 9 years of age showed a significant reduction in adult female monkeys born in 2008, relative to monkeys born in 2009 (Figure 4). For study offspring, there were no significant differences when comparing mixed gender animal groups. However, exclusive comparison of male offspring indicated a reduced rate of body weight gain from birth year through 2-3 years of age from animals born to adult female monkeys exposed to early life wildfire smoke $PM_{2.5}$ (Figure 5A). In contrast, female offspring showed no significant differences in weight gain between study groups (animals born to 2008 females versus animals born to 2009 females) (Figure 5B).

| Date of Birth | Gender | Age at June 24 (days) | Weight (kg) |
|---|---|---|---|
| 2/25/08 | F | 120 | 10.12 |
| 2/25/08 | F | 120 | 7.03 |
| 2/28/08 | F | 117 | 9.25 |
| 3/06/08 | F | 110 | 12.01 |
| 3/10/08 | F | 106 | 7.57 |
| 3/18/08 | F | 98 | 7.59 |
| 3/24/08 | F | 92 | 6.02 |
| 3/25/08 | F | 91 | 6.1 |
| 3/25/08 | F | 91 | 7.34 |
| 3/26/08 | F | 90 | 6.57 |
| 3/27/08 | F | 89 | 8.47 |
| 3/28/08 | F | 88 | 8.2 |
| 3/31/08 | F | 85 | 7.32 |
| 4/01/08 | F | 84 | 8.87 |
| 4/02/08 | F | 83 | 7.87 |
| 4/09/08 | F | 76 | 10.89 |
| 4/10/08 | F | 75 | 5.6 |
| 4/10/08 | F | 75 | 7.4 |
| 4/12/08 | F | 73 | 7.5 |
| 4/12/08 | F | 73 | 8.55 |
| 4/15/08 | F | 70 | 10.48 |
| 4/19/08 | F | 66 | 9.46 |
| 4/28/08 | F | 57 | 7.82 |
| 5/03/08 | F | 52 | 7.49 |
| 5/09/08 | F | 46 | 7.54 |
| 5/15/08 | F | 40 | 9.44 |
| 5/22/08 | F | 33 | 7.99 |
| 5/22/08 | F | 33 | 8.96 |
| 5/24/08 | F | 31 | 7.79 |
| 6/12/08 | F | 12 | 10.6 |

**Table 1. Age and weight distribution of CNPRC female monkeys born in 2008.** The average weight for evaluated female monkeys born in 2008 was 8.26 kg $\pm$ 1.51 SD.

| Date of Birth | Gender | Age at June 24 (days) | Weight (kg) |
|---|---|---|---|
| 2/20/14 | F | 2.72 | 4.52 |
| 3/27/14 | M | 2.66 | 5 |
| 4/14/14 | F | 2.55 | 3.19 |
| 4/18/14 | F | 2.43 | 3.75 |
| 5/05/14 | F | 2.55 | 5.05 |
| 5/06/14 | M | 2.15 | 3.63 |
| 5/10/14 | F | 2.5 | 3.29 |
| 6/10/14 | F | 2.09 | 2.82 |
| 3/7/15 | F | 1.66 | 2.01 |
| 3/21/15 | F | 1.64 | 1.86 |
| 3/22/15 | F | 1.46 | 2.86 |
| 3/26/15 | F | 1.62 | 1.93 |
| 3/26/15 | F | 1.61 | 2.36 |
| 3/29/15 | M | 1.62 | 3.14 |
| 3/29/15 | M | 1.79 | 3.1 |
| 4/7/15 | F | 1.28 | 1.56 |
| 4/9/15 | M | 1.57 | 3.55 |
| 4/10/15 | M | 1.76 | 3.26 |
| 4/18/15 | M | 1.6 | 2.25 |
| 4/28/15 | M | 1.34 | 2.18 |
| 4/28/15 | F | 1.57 | 2.54 |
| 5/2/15 | M | 1.39 | 2.51 |
| 5/2/15 | M | 1.29 | 1.29 |
| 5/2/15 | M | 1.56 | 2.9 |
| 5/7/15 | M | 1.18 | 1.62 |
| 5/11/15 | M | 1.5 | 2.48 |
| 5/12/15 | F | 1.19 | 1.3 |
| 5/12/15 | F | 1.5 | 1.6 |
| 5/13/15 | M | 1.49 | 2.16 |
| 5/17/15 | F | 1.48 | 2.26 |

**Table 2. Age and weight distribution of offspring from CNPRC female monkeys born in 2008.** The average weight for evaluated offspring was 2.73 kg $\pm$ 0.99 SD.

| Date of Birth | Gender | Age at June 24 (days) | Weight (kg) |
|---|---|---|---|
| 2/24/09 | F | 120 | 7.87 |
| 3/03/09 | F | 113 | 7.58 |
| 3/06/09 | F | 110 | 9.83 |
| 3/13/09 | F | 103 | 10.46 |
| 3/15/09 | F | 101 | 9.12 |
| 3/25/09 | F | 91 | 7.9 |
| 3/27/09 | F | 89 | 9.72 |
| 3/27/09 | F | 89 | 6.61 |
| 3/29/09 | F | 87 | 8.1 |
| 4/03/09 | F | 82 | 10.74 |
| 4/08/09 | F | 77 | 8.04 |
| 4/09/09 | F | 76 | 8.24 |
| 4/10/09 | F | 75 | 12.27 |
| 4/10/09 | F | 75 | 6.72 |
| 4/13/09 | F | 72 | 9.21 |
| 4/16/09 | F | 69 | 9.93 |
| 4/18/09 | F | 67 | 12.35 |
| 4/19/09 | F | 66 | 9.54 |
| 4/22/09 | F | 63 | 8.4 |
| 4/23/09 | F | 62 | 8.61 |
| 4/27/09 | F | 58 | 8.83 |
| 5/06/09 | F | 49 | 8.72 |
| 5/23/09 | F | 32 | 8.63 |
| 5/25/09 | F | 30 | 6.17 |
| 5/26/09 | F | 29 | 12.17 |
| 6/01/09 | F | 23 | 7.91 |
| 6/02/09 | F | 22 | 10.17 |
| 6/03/09 | F | 21 | 8.79 |
| 6/05/09 | F | 19 | 9.91 |
| 6/06/09 | F | 18 | 9.05 |
| 7/07/09 | F | -13 | 9.34 |

**Table 3. Age and weight distribution of CNPRC female monkeys born in 2009.** The average weight for evaluated female monkeys born in 2009 was 9.06 kg $\pm$ 1.52 SD.

| Date of Birth | Gender | Age at June 24 (days) | Weight (kg) |
|---|---|---|---|
| 2/26/15 | M | 2.37 | 3.83 |
| 3/18/15 | M | 2.57 | 3.5 |
| 4/2/15 | M | 2.43 | 3.12 |
| 4/7/15 | M | 2.17 | 3.6 |
| 4/15/15 | F | 2.24 | 2.87 |
| 4/19/15 | M | 2.45 | 3.19 |
| 4/20/15 | F | 2.37 | 3.85 |
| 4/21/15 | F | 2.47 | 3.16 |
| 4/22/15 | F | 2.61 | 3.42 |
| 5/2/15 | M | 2.42 | 3.9 |
| 5/5/15 | M | 2.40 | 3.39 |
| 5/8/15 | F | 2.56 | 3.4 |
| 5/9/15 | F | 2.25 | 2.65 |
| 5/9/15 | M | 2.39 | 3.05 |
| 5/11/15 | M | 2.40 | 4.19 |
| 6/11/15 | M | 2.31 | 3.2 |
| 6/15/15 | F | 2.45 | 2.99 |
| 6/18/15 | M | 2.34 | 3.87 |
| 2/20/16 | F | 1.64 | 1.75 |
| 3/2/16 | F | 1.74 | 2.55 |
| 3/19/16 | M | 1.47 | 1.79 |
| 3/28/16 | M | 1.56 | 2.56 |
| 3/31/16 | M | 1.32 | 1.42 |
| 4/2/16 | F | 1.29 | 1.44 |
| 4/16/16 | M | 1.20 | 2.14 |
| 5/1/16 | F | 1.27 | 1.63 |
| 5/5/16 | F | 1.18 | 1.78 |
| 5/6/16 | F | 1.10 | 1.52 |
| 5/7/16 | F | 1.10 | 1.94 |
| 5/30/16 | F | 1.36 | 1.79 |
| 6/1/16 | F | 1.38 | 2.03 |

**Table 4. Age and weight distribution of offspring from CNPRC female monkeys born in 2008.** The average weight for evaluated offspring was 2.76 kg $\pm$ 0.86 SD.

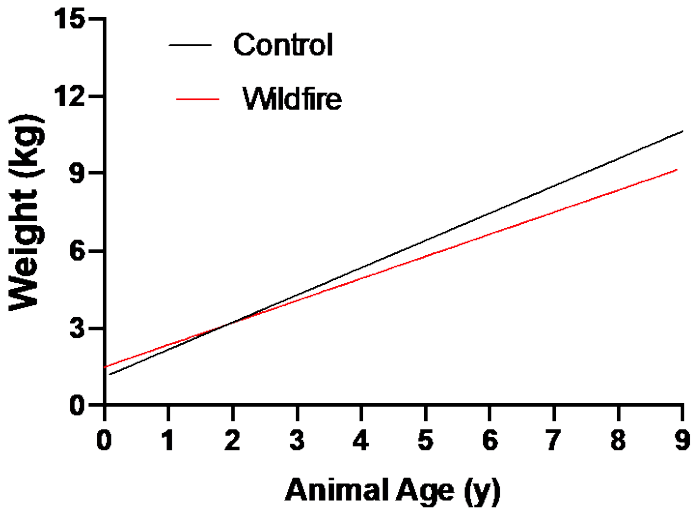


**Figure 4. Linear regression analysis of body weight data between birth and nine years of age collected from adult female monkeys exposed to early life wildfire smoke $PM_{2.5}$.** Adult female monkey weight gain from birth through nine years of age. Linear regression analysis of weight gain from birth through nine years of age for study adult female monkeys. $R^2$ values were 0.9010 for control adults and 0.7964 for wildfire adults. Slopes between exposed and control animals were significantly different with p-value <0.0001.

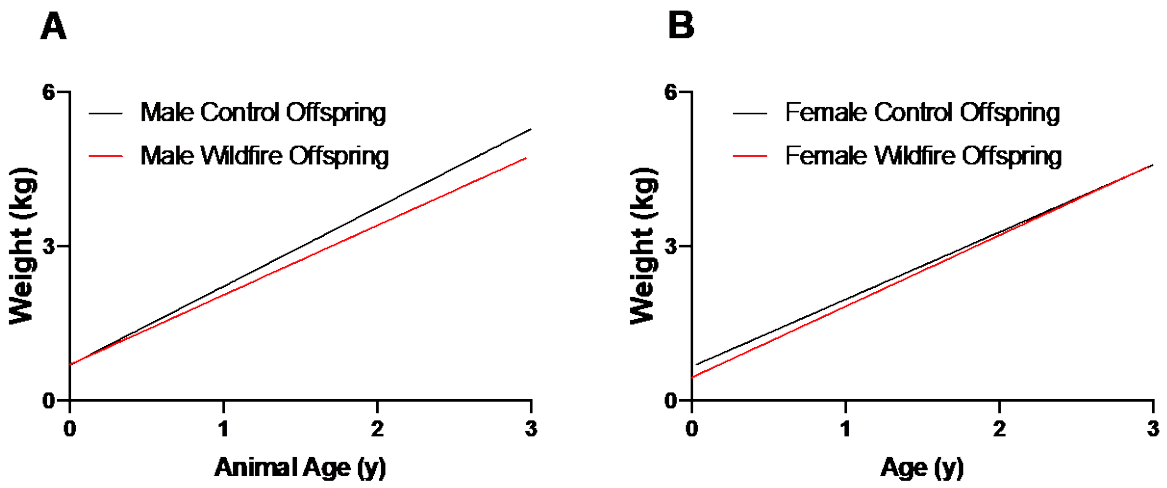


**Figure 5. Linear regression analysis of body weight data between birth and three years of age collected from offspring of adult female monkeys exposed to early life wildfire smoke $PM_{2.5}$.**
A. Linear regression analysis of weight gain from birth through three years of age for male offspring associated with study adult female monkeys. $R^2$ values were 0.8508 for offspring from exposed females and 0.9370 for offspring from control females. Slopes between control offspring and wildfire offspring were significantly different with p-value = 0.0013 B. Linear regression analysis of weight gain from birth through three years of age for female offspring associated with enrolled adult female monkeys. $R^2$ values were 0.9209 for offspring from exposed females and 0.9273 for offspring from control females. Slopes between offspring from exposed females and offspring from control females were not significantly different with p-value = 0.0744.

Summary of Cumulative Exposure

To determine the lifetime exposure profiles of the 2008 and 2009 animal cohorts, publicly available data was collected from a California Air Resources Board monitoring station, located on the UC Davis campus 2.7 miles from the CNPRC. The cumulative amount of $PM_{2.5}$ and ozone experienced by each study animal over six-month intervals was quantified, starting from date of birth and terminating at eight years of age. The CNPRC electronic medical record system was used to determine the daily location (outdoor versus indoor) of each study animal during the eight-year period of assessment. Air quality data on dates spent indoors in HEPA filtered housing for observation and veterinary intervention purposes were excluded from animals on an individual basis. Adult female monkeys exposed to early life wildfire smoke $PM_{2.5}$ (born in 2008) experienced approximately 9.6 more days with $PM_{2.5}$ levels greater than the National Ambient Air Quality Standards (NAAQS) set for $PM_{2.5}$ (35 $\mu g/m^3$ for a 24-h average, EPA 40 CFAR 50, Figure 6A) than adult female monkey controls (born in 2009). Exposed animals experienced a greater peak in air pollutant levels with the maximum daily value for $PM_{2.5}$ reaching 76 $\mu g/m^3$ during the first 6 months of life (Figure 6B), approximately 3.8 times greater than the maximum daily value experienced by control animals. Cumulatively, across an eight-year lifespan, the adult female monkeys exposed to early life wildfire smoke $PM_{2.5}$ experienced approximately 8% more $PM_{2.5}$ than control animals (Figure 6C, 6D).

Analysis of ambient ozone concentrations revealed few differences in peak, average, or cumulative exposure between wildfire-exposed and control cohorts (Figure 7). However, adult female monkeys exposed to early life wildfire smoke $PM_{2.5}$ experienced approximately 5.1 more days with ozone concentrations above the NAAQS set for ozone (0.070 ppm/8-hour average, EPA 40 CFAR 50, Figure 7A). The age of each adult study animal at peak 24-hour $PM_{2.5}$ (Figure 8A) and ozone (Figure 8B, 8C) exposure was calculated; adult female monkeys exposed to early life wildfire smoke $PM_{2.5}$ were found to be significantly younger at peak exposure concentrations. Exposed animals were approximately 2.5 months of age at peak $PM_{2.5}$ and ozone exposures, whereas control animals did not experience peak $PM_{2.5}$ or ozone exposure until approximately 2-3 years of age. Compared to controls, adult female monkeys exposed to early life wildfire smoke $PM_{2.5}$ experienced greater levels of $PM_{2.5}$ with little difference in ozone exposure during early life, and relatively similar levels of $PM_{2.5}$ and ozone exposure throughout adolescence (three years of age) and adulthood (eight years of age). A significant difference in the age at peak exposure between offspring of adult female monkeys was also observed, however it should be noted that the age gap between offspring groups was less when compared with adults (approximately 1 year for $PM_{2.5}$ and 6 months for ozone)(Figure 8D, 8E).

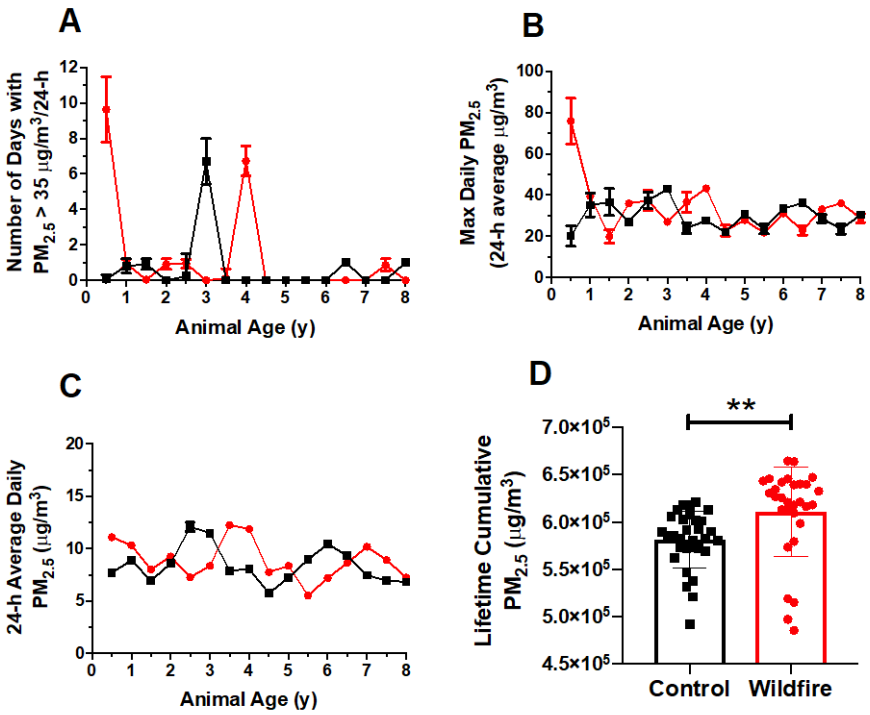


**Figure 6. Quantification of $PM_{2.5}$ exposure for adult female monkeys exposed to early life wildfire smoke $PM_{2.5}$.** Air quality data was compiled for individual adult female animals from birth to eight years of age and adjusted for time spent indoors. The number of days exceeding the NAASQ standard for $PM_{2.5}$ (>35 μg/$m^3$/24-h) was determined for each six month interval period (A). Similarly, the highest recorded level for $PM_{2.5}$ (B) and average daily $PM_{2.5}$ concentrations (C) were determined. Lifetime cumulative $PM_{2.5}$ exposure was calculated as the sum of ambient hourly measurements from birth through eight years of age (D). N=30 adult female monkeys per group. **p<0.01

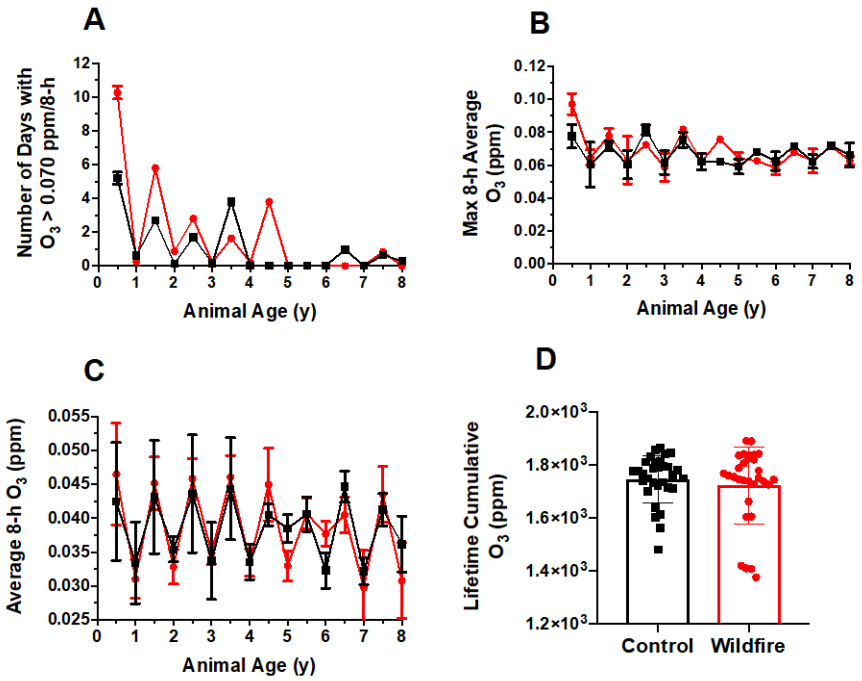


**Figure 7. Quantification of ozone ($O_3$) exposure for adult female monkeys exposed to early life wildfire smoke $PM_{2.5}$.** Local air quality was recorded from 2008 to 2018 by a California Air Resources monitoring station. Air quality data was compiled for individual adult female animals from birth to eight years of age and adjusted for time spent indoors. The number of days exceeding the NAASQ standard for $O_3$ (>0.070 ppm/8-h) was determined for each six month interval period. Similarly, the highest recorded level for $O_3$ (B) and 8-h average $O_3$ concentrations (C) were determined. Lifetime cumulative $O_3$ exposure was calculated as the sum of ambient hourly measurements from birth through eight years of age. N=30 adult female monkeys per group.

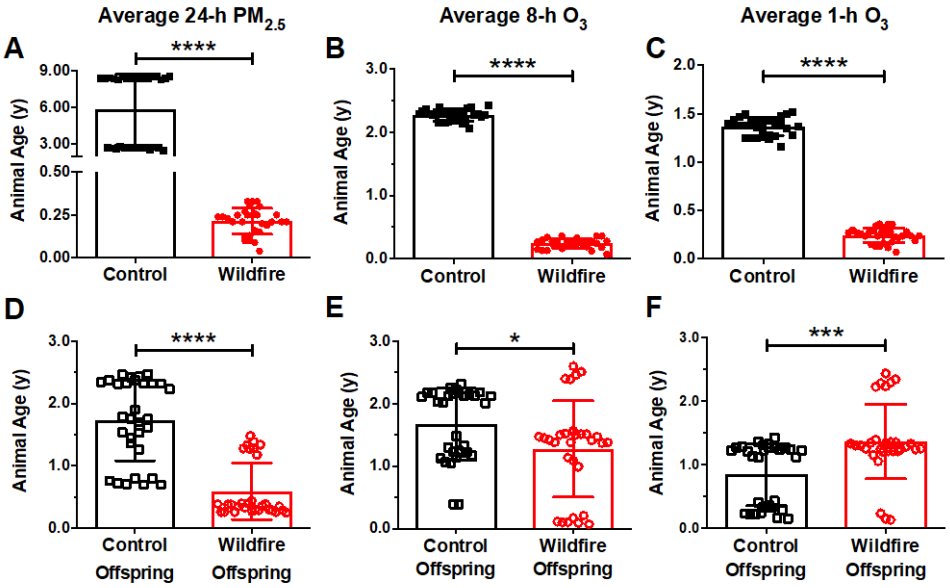


**Figure 8. Age at peak wildfire smoke $PM_{2.5}$ exposure for adult female monkeys and associated offspring.** Age of exposed and control adult female monkeys at peak 24-h average $PM_{2.5}$ (A), 8-h average $O_3$ (B), and 1-h average $O_3$ (C) exposure from birth to eight years of age. Age of associated offspring at peak 24-h average $PM_{2.5}$ (D), 8-h average $O_3$ (E), and 1-h average $O_3$ (F) exposure. N=30 adult female monkeys per group. N=30 offspring of female monkeys per groups.

Analysis of Immune Profiles

To determine whether the immune response to an infectious challenge might be compromised in animals exposed to wildfire smoke during early life, an *in vitro* PBMC culture assay established from peripheral blood samples obtained from study animals was used to assess for the ability of circulating immune cells to respond to a microbial ligand challenge as previously described (33). Cytokine responses following 24-hour treatment of PBMC cultures with the Toll-like receptor 4 agonist, LPS, were measured with a non-human primate 29-plex Luminex panel. LPS is a component of gram-negative bacteria cell walls and can rapidly elicit an innate immune response that mimics a bacterial infection both *in vivo* and *in vitro*. Of the 29 cytokines tested by the panel, 12 were detectable in culture supernatants from study animal PBMC. Of the 12 detectable cytokines, 6 were determined to be significantly different between exposed (born in 2008) and control (born in 2009) adult animals at eight years of age. All detectable cytokines were classically associated with innate immune responses that are rapidly elicited following any microbial infection, specifically of monocyte/macrophage origin. Adaptive immune cytokines that are primarily produced by immune cells of lymphoid origin such as IFNγ, IL-5, IL-4, and IL-10 registered below the limit of detection for this assay (data not shown). Adult female monkeys that were exposed to early life $PM_{2.5}$ displayed significantly decreased basal levels of TNF-α, MCP-1, and MIF relative to their control counterparts (Figure 8B, 8D, 8E). In response to LPS treatment, exposed adult animals displayed lower levels of MIP-1β and IL-1RA relative to controls (Figure 8A, 8F). Levels of IL-1β did not change significantly in exposed adult animals; however, they tended to be higher than controls (data not shown). RANTES was significantly elevated in response to LPS in exposed animals compared to controls (Figure 8C). There was a trend toward higher concentrations of GM-CSF, IL-6, IL-8, and MIP-1α in response to LPS from PBMC cultures of exposed animals, however these results were not significant (data not shown). Cytokines that were differentially expressed in PBMC cultures derived from adult female monkeys exposed to early life $PM_{2.5}$ have pleotropic properties, however reduced expression is suggestive of an attenuated immune response to a potential microbial challenge.

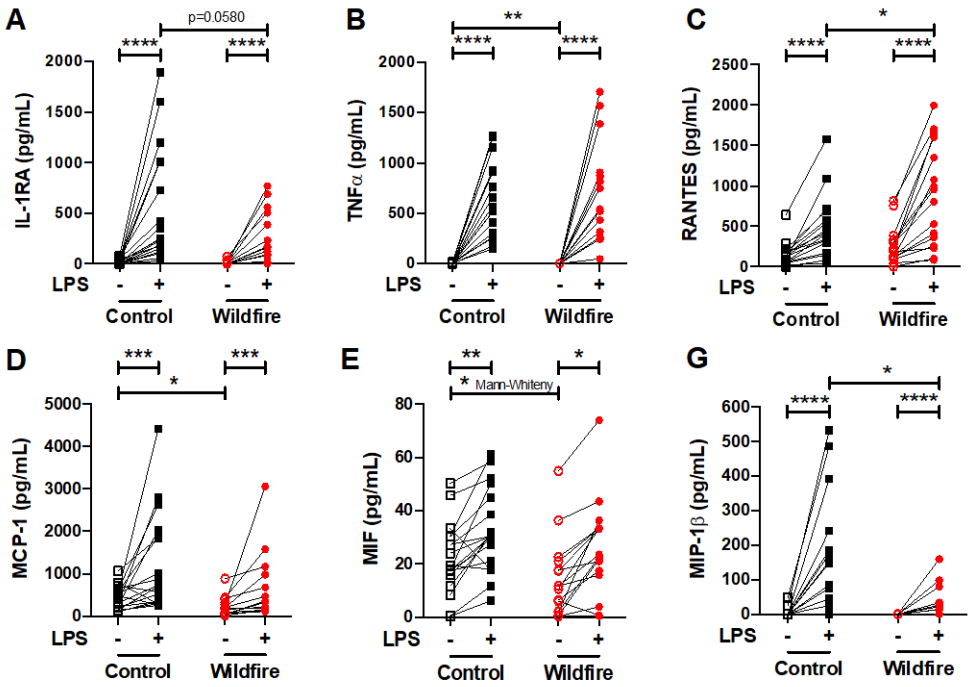


**Figure 9. Early life wildfire smoke $PM_{2.5}$ exposure is associated with altered systemic inflammatory responses.** PBMCs isolated from exposed (N=14) or control animals (N=14) were untreated or stimulated for 24 hours with 50 ng/mL of LPS. Supernatant was collected from media or LPS stimulated PBMC and analyzed for expression of 29 different cytokines by Luminex. Absolute concentrations for significantly differentially regulated cytokines: IL-1RA (A), TNFα (D), RANTES (C), MCP-1 (D), MIF (E), and MIP-1β (G) are shown above. * p-value < 0.05. ** p-value < 0.005. *** p-value < 0.001. **** p-value < 0.0001

To understand how wildfire smoke exposure might alter systemic immunity, levels of IL-8 protein and C-reactive protein (CRP) were measured in the serum of both adult female monkeys and their associated offspring using standard ELISA methods. Adult female monkeys exposed to wildfire smoke $PM_{2.5}$ as infants presented with significantly lower levels of serum CRP and no significant change in serum IL-8 (Figures 10A,10B). Offspring of adult female monkeys exposed to wildfire smoke $PM_{2.5}$ as infants presented with significantly lower serum IL-8 and no significant change in serum CRP (Figures 10C, 10D) when compared to control offspring. To determine if humoral changes could be detected in association with wildfire smoke exposure, anti-tetanus toxoid specific IgG (α-TT-IgG) was also measured in serum samples. All monkeys in the CNPRC colony are vaccinated against tetanus starting in the first year of life and receive frequent boosters. No significant difference in α-TT-IgG was detected between exposed or control adult female monkeys and no significant difference was found in associated offspring groups (Figure 11).

To assess for additional multigenerational effects of wildfire smoke exposure, we compared cytokine responses in PBMC cultures following *in vitro* challenge with LPS. We have previously reported that PBMC obtained from animals at 3 years post wildfire smoke exposure showed significantly different levels of expression in IL-6 and IL-8 in response to LPS (33). We found that wildfire smoke-exposed adult female monkeys and their associated offspring produced significantly greater levels of IL-6 in response to 50 ng/mL of LPS compared to age-matched controls (Figures 12A, 12C). Wildfire smoke-exposed adult female monkeys also produced greater levels of IL-8 in response to 5 or 50 ng/mL of LPS; however, their associated offspring displayed reduced production of IL-8 at 50 ng/mL of LPS compared to age-matched controls (Figure 12B, 12D).

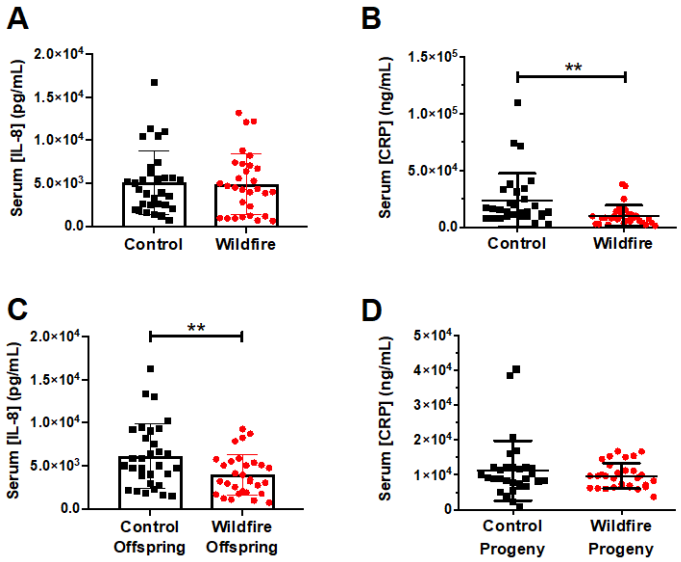


**Figure 10. Association of systemic innate immune parameters with early life wildfire smoke $PM_{2.5}$ exposure.** Serum IL-8 (A, C) and serum C-reactive protein (CRP) (B, D)) were measured by ELISA for adult female monkeys (A, B; N=30 per group) and their associated offspring (C, D; N=30 per group). ** p-value < 0.005.

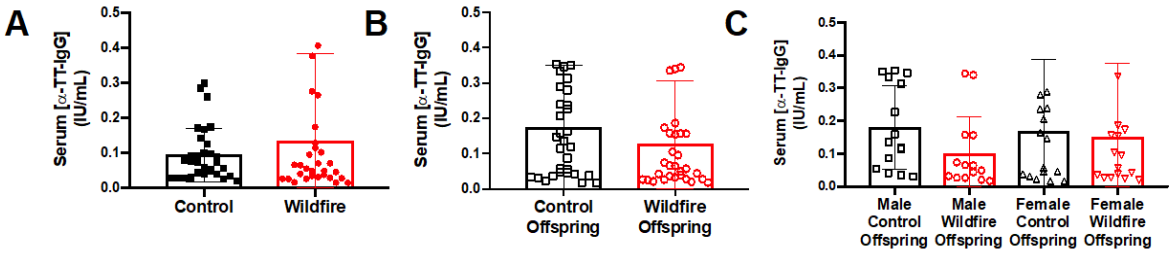


**Figure 11. Effect of early life wildfire smoke $PM_{2.5}$ exposure on humeral immunity against tetanus toxoid in adult female monkeys and associated offspring.** Serum anti-tetanus toxoid IgG (α-TT-IgG) was measured by ELISA for exposed and control adult female monkeys (N=30 per group) (A) and their associated offspring (N=30 per group)(B, C). Serum tetanus toxoid IgG levels were deconvoluted in associated offspring by sex (C).

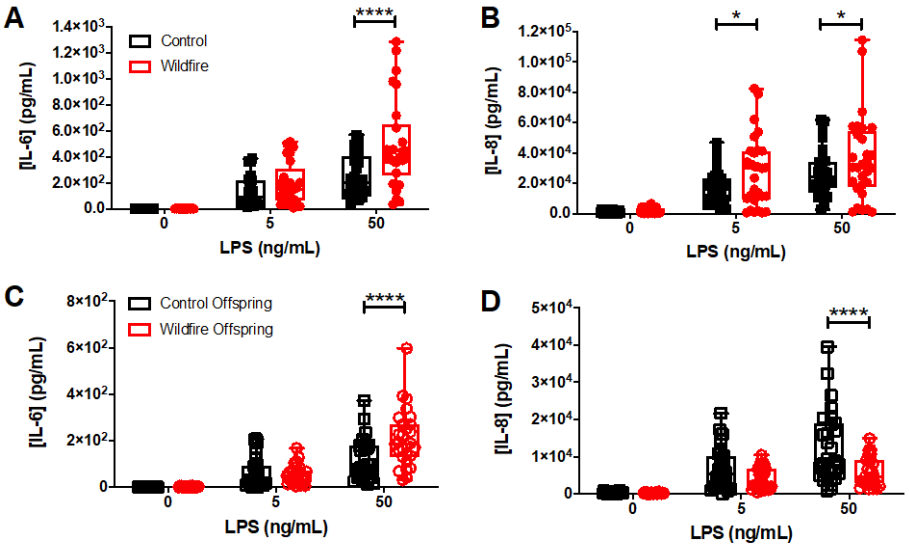


**Figure 12. Association of dysregulated innate immune responses with early life wildfire smoke $PM_{2.5}$ exposure in adult female monkeys and offspring.** PBMC from exposed or control adult female monkeys (N=30 per group) and their associated offspring (N=30 per group) were stimulated with LPS for 6 hour. Supernatant from cell cultures was collected and measured for IL-6 (A and B) and IL-8 (C and D) by ELISA . ** p-value < 0.005.

Analysis of Lung Structure and Function

A subset of eight-year-old adult female monkeys exposed to early life wildfire smoke $PM_{2.5}$ and control animals were randomly selected for evaluation by HRCT. HRCT imaging can be a more clinically relevant methodology to evaluate anatomical and physiological changes present as a result of antecedent wildfire exposure due to the more frequent use of this modality to assess human patients for chronic lung diseases such as pulmonary fibrosis and chronic obstructive pulmonary disease. Functional respiratory imaging (FRI) technology recently developed by an industry partner was used to quantitatively determine if any differences in pulmonary physiology existed between the two cohorts.

Results from HRCT analysis indicated that compared to controls, adult female monkeys exposed to early life wildfire smoke $PM_{2.5}$ had significantly ($p < 0.001$) reduced lung volumes, inspiratory capacities, and ventilation perfusion ratios as adults (Figures 13A, 13B, 13C, respectively). Ventilation perfusion ratios provide information on the quantity of air that reaches alveoli (ventilation) and the amount of blood that reaches the capillaries of the alveoli (perfusion); a lower ventilation perfusion ratio indicates impaired gas exchange. The average total lung volume of exposed animals was approximately 20% lower than age-matched controls at total lung capacity (TLC, Figure 13A), which is indicative of the volume of lungs at maximal inflation. A 20% reduction in TLC may be indicative of an early lung stage lung disease but is unlikely to be symptomatic; a greater than 50% reduction is often seen in human patients seeking medical treatment. At functional residual capacity (FRC), which is the volume of lungs at end-expiratory position, little difference was detected between the two groups (data not shown). The left lower lobes displayed the greatest change in lung volume and ventilation perfusion (Figures 13D and 13F, respectively). Exposed animals presented with approximately 28% less volume in the lower left lobes (Figure 13D) and a 55% reduction in ventilation perfusion ratio (Figure 13F) compared to controls. In contrast, the right middle lobe of exposed animals appeared to display the greatest reduction in inspiratory capacity, approximately 60% relative to controls (Figure 13E). Reduced gas exchange as not directly tested, however given the reduction in ventilation perfusion ratios (Figure 13C, 13F), it is likely that exposed animals have impaired gas exchange capacity.

Further analysis of the blood vessel density revealed an overall increase in blood vessel volume for wildfire exposed animals relative to controls (Figure 14A). The left lung displayed the greatest change in blood vessel density, while the distal right lung had greater blood vessel density than the right proximal lung (Figure 14C). The link between enhanced lung angiogenesis and respiratory disease is somewhat controversial in the literature, in part due to limitations in quantifying blood vessel density by imaging methods. Exposed animals also presented with evidence of increased specific airway radius relative to control animals (Figure 14B). Greater increases in airway radius were detected in lower lung lobes, compared to upper lung lobes of exposed animals (Figure 14D), a phenotype suggesting that airflow is diverted into the distal conducting airways as a compensatory mechanism for reduced expansion capability of alveoli.

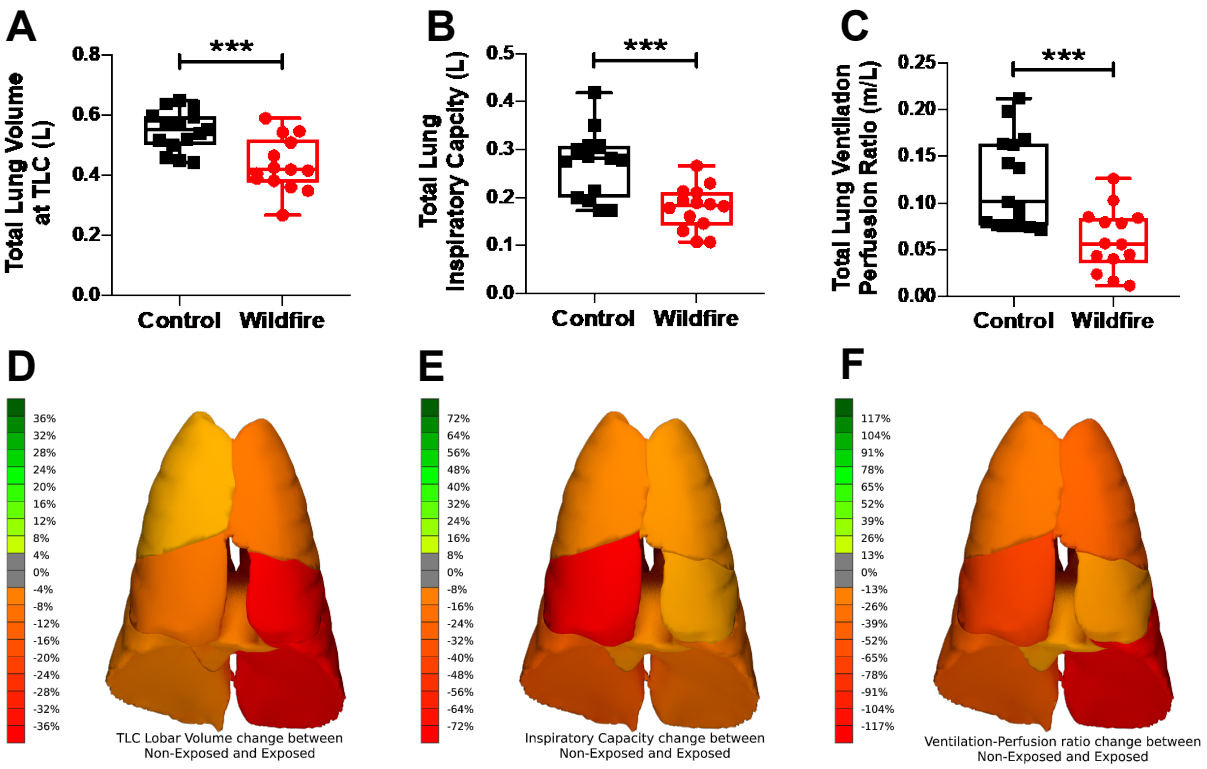


**Figure 13. High resolution computed tomography thoracic wall imaging of adult female monkeys exposed to early life wildfire smoke $PM_{2.5}$.**
CT images at total lung capacity (TLC) and functional residual capacity (FRC) were analyzed to determine total lung volume (A), inspiratory capacity (B), and ventilation perfusion ratios (C). Box plots show the average values for lung volume, inspiratory capacity and ventilation perfusion rations +/- standard deviation of n=14 monkeys per group. Changes to lobe specific sections are represented as the average as percent deviation by wildfire exposed monkeys compared to controls for lobar volume (D), inspiratory capacity (E), and ventilation perfusion ratio (F). Red indicated a decrease, whereas green indicated an increase in the average values for wildfire exposed animals compared to controls. *** p-value < 0.001 by Student's t test.

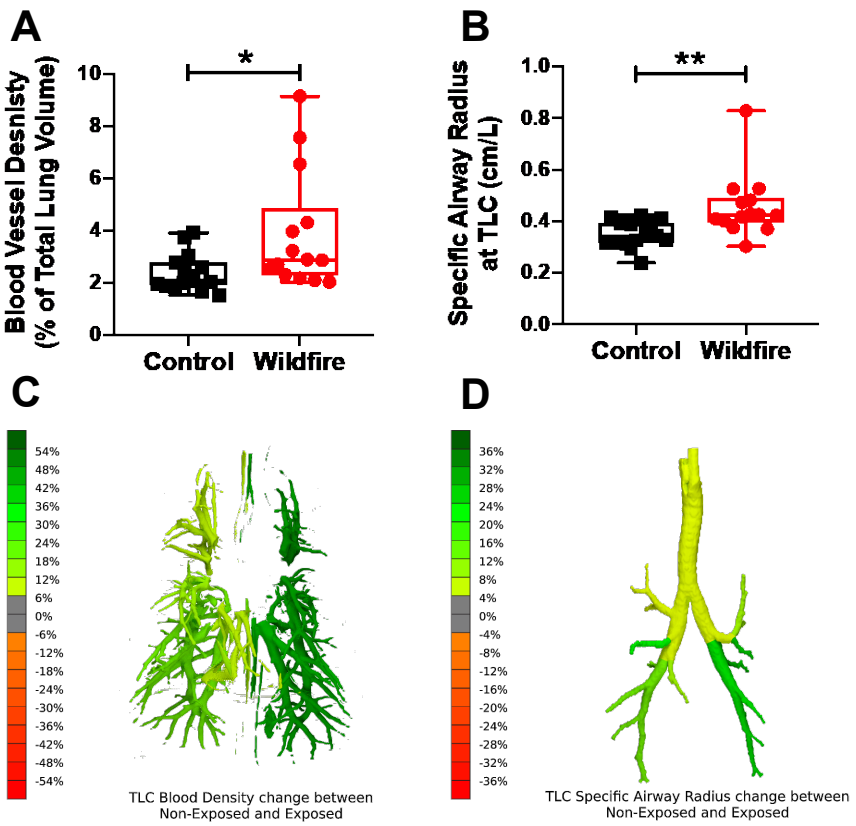


**Figure 14. Early life wildfire smoke $PM_{2.5}$ exposure is associated with increased blood vessel density and airway radius in adult female monkeys.** CT images at total lung capacity (TLC) and functional residual capacity (FRC) were analyzed to determine total blood vessel density (A) and specific airway radius (B). Changes to lobe specific sections are represented as the average as percent deviation by exposed monkeys compared to controls for blood vessel density (C) and specific airway radius (D). Red indicated a decrease, where as green indicated an increase in the average values for exposed animals compared to controls. N=14 adult female monkeys were sampled for each group. * p-value < 0.05. ** p-value < 0.01.

## Discussion

Long term respiratory health effects of early life pollution exposures have been documented in human epidemiology, however there are limited data on extreme air quality events associated with chronic disease. Bharadwaj et. al. 2016 reported that early life exposure to the Great Smog of London in 1952 significantly enhanced the likelihood of childhood and adult asthma, suggesting that an extreme air quality event can result in health effects that persist with maturity (10). Our investigation of immune and respiratory health outcomes in a nonhuman primate cohort offers a unique opportunity to investigate the long-term impact of an acute ambient air pollution event (wildfire smoke $PM_{2.5}$) in a laboratory animal housed in a well-characterized environment at the CNPRC.

We have previously reported that peripheral blood cultures from juvenile animals exposed to early life wildfire smoke $PM_{2.5}$ displayed reduced IL-6 and IL-8 responses to LPS (25). In contrast with our findings in juvenile animals, we have observed the IL-6 and IL-8 response to LPS was increased in peripheral blood cultures established from adult female monkeys. While the mechanism for the divergent outcome is unknown, systemic shifts in cytokine production are inherent with age, a phenomenon that has been recently described as inflammaging. Inflammaging is defined as the tendency to increase cytokine production in response to similar stimuli with age; presently however, the mechanism driving this observation is still unclear (34, 35). Our data suggest that early life responses proceeding wildfire smoke exposure may initially attenuate IL-6 and IL-8 expression, but progressive lead to augmented production during adulthood. Using a multiplex antibody approach, we also detected changes in numerous cytokines classically associated with monocytes and macrophages. Our results from peripheral blood assays suggest that wildfire smoke $PM_{2.5}$ exposure might alter monocyte/macrophage function, which could contribute to disease onset in the respiratory tract.

HRCT findings in this study showed similar features of idiopathic pulmonary fibrosis (IPF) in humans. However, it should be noted that exposed animals lacked several key features associated with clinical diagnosis of IPF. In particular, no changes in internal airflow distribution or evidence of brown glass opacity, such as honeycombing or traction bronchiectasis were detected (data not shown) (36). The average age of study animals at the time of assessment was 8.5 years, which is comparable to a human subject of approximately 30 years (37). IPF patients are often diagnosed after 50 years of age, therefore it is possible that our findings in young adult monkeys may be reflective of early stage events (36, 38). Due to the non-invasive nature of our study, we did not collect an airway biopsy from these animals and therefore lack histological evidence of fibrosis. However, based upon HRCT analysis, it may be speculated that early life wildfire smoke $PM_{2.5}$ exposure may have elicited development of a fibrotic phenotype.

Our HRCT results corroborate with pulmonary function testing results from three year old adolescent female monkeys described in Black et al. 2017 (25). It should be noted that these studies were conducted on two different groups of female monkeys selected from a large cohort of CNPRC animals exposed to wildfire smoke $PM_{2.5}$ in 2008. Both studies detected decreased pulmonary volumes and inspiratory capacities. In particular, Black et al. noted increased compliance in exposed female monkeys (25). Our HRCT analysis could not directly calculate compliance, however increased specific

airway radius and blood vessel density were detected. Pressure generated by the diaphragm during inhalation could have been redistributed to the conducting airways due to increased fibrosis at the alveoli. We postulated that increased blood vessel density might have occurred as a physiological compensatory mechanism to reduced inspiratory capacity. Indeed, previous studies have reported increased angiogenesis in IPF patients with increased IL-8 (39). Our results are consistent with two human epidemiological studies that have demonstrated an association between early life exposures to high $PM_{2.5}$ and reduced FVC measurements by spirometry later in life (17, 18).

There are several limitations to this study. This was a retrospective study and as such had confounding variables we could not account for. Our previous study in Black et al. demonstrated that adult monkeys co-housed with infant monkeys and exposed to similar levels of $PM_{2.5}$ from 2008 Trinity/Humboldt County wildfire smoke presented no change in cytokine synthesis three years after the exposure event. These findings suggested that adult subjects are less sensitive to wildfire smoke-mediated immunotoxicity, which is supported by an epidemiologic study in human cohorts with traffic related air pollution (18), where exposures past the first year of life had no association with reduced $FEV_1$. Dysregulation of immune responses and onset of pulmonary pathology in adulthood are therefore likely to be influenced by early life exposures. However, we cannot rule out the possibility that multiple exposure episodes may still be required for final disease onset. It is also important to consider that the rhesus macaques in this study are housed in outdoor breeding colonies and as such, have received a significantly higher exposure in comparison with the human population that can remain indoors to limit exposure during a wildfire event. Lastly, although very close to human physiology, rhesus monkeys still age at an expedited rate (37). The magnitude and duration of exposure may need to be greater to elicit true effects in humans. With wildfires increasing in frequency and severity, it is likely that the observations recorded in our non-human primate model may become increasing applicable to infants living through these wildfire episodes.

To the best of our knowledge, this is the first study to report wildfire smoke mediated multigenerational effects on unexposed offspring. The altered immune response of exposed animals was associated with altered immune responses of offspring. Our data suggested that early life wildfire smoke $PM_{2.5}$ persistently dysregulated immune responses to LPS and elicited a fibrotic lung phenotype that was detectable during adolescence (25). Previous studies have suggested that pulmonary fibrosis may be a consequence of increased inflammation and tissue repair in the lung (40). Early life wildfire smoke $PM_{2.5}$ exposure could epigenetically modify immune responses in exposed animals and transfer phenotypes to subsequent generations. However, more studies are required to determine if this is a driving cause of the fibrotic phenotype. It is also notable that both adult female monkeys and their respective offspring showed significant differences in weight gain over time. While we were unable to further investigate the mechanisms of reduced weight in exposed animals, it may be speculated that differences in metabolic pathways or microbiome would be detected.

In summary, we have demonstrated that early life exposure to ambient air pollution produced by wildfires is highly associated with persistent changes in immune responses and airway physiology. Our results suggest that exposure to wildfire smoke during infancy

can result in structural changes to the lung and an altered immune response in adulthood. Moreover, these changes can have multigenerational effects on offspring.

## Summary and Conclusions

The objective of this study was to determine the impact of early life $PM_{2.5}$ exposure from wildfire smoke on parameters of immunity and respiratory structure that influence responses to infectious disease and lung function in adulthood. To complete this objective, we investigated a cohort of California National Primate Research Center outdoor housed female adult rhesus monkeys that were born within three months prior to the Trinity and Humboldt County wildfires of June/July 2008. We also assessed whether offspring from wildfire smoke-exposed female monkeys showed evidence of immune dysregulation. We hypothesized that wildfire smoke $PM_{2.5}$ exposure during early life would result in an altered innate immune response to infection and lung function decrements in adult animals. Our hypothesis was based upon previously published findings in wildfire smoke $PM_{2.5}$ exposed adolescent animals. To test this hypothesis, we (1) evaluated the immune response to microbial ligands in blood samples and (2) obtained lung structural data using *in vivo* imaging methods. Our data show evidence of persistent immune dysregulation in adult monkeys that were previously exposed to wildfire smoke $PM_{2.5}$ as infants. Following treatment with microbial ligands, peripheral blood cultures from wildfire smoke-exposed monkeys showed increased expression of proinflammatory cytokines relative to controls. Peripheral blood cultures from offspring of adult female monkeys exposed to early life wildfire smoke $PM_{2.5}$ also displayed significantly different responses to microbial challenge relative to control counterparts. HRCT scans from adult female monkeys exposed to early life wildfire smoke $PM_{2.5}$ showed reductions in lung volume, inspiratory capacity, and ventilation perfusion relative to adult female monkey controls. An increase in specific airway radius and blood vessel density was also detected in HRCT scans from adult female monkeys exposed to early life wildfire smoke $PM_{2.5}$. Our findings demonstrate that early life ambient wildfire smoke $PM_{2.5}$ exposure may be associated with immune and pulmonary responses that persist into adulthood. We further provide evidence of multigenerational transmission of immune dysregulation as a result of maternal wildfire smoke exposure. Because we evaluated the effect of an ambient pollutant exposure on a population of monkeys living outdoors, the amount of $PM_{2.5}$ inhaled by individual animals may vary and potentially confound our findings. Further research is needed to determine whether humans with similar exposures to wildfire smoke $PM_{2.5}$ can exhibit a similar health profile.

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