

# **Effects of Nitrogen Dioxide on Non-Specific Airway Inflammation in Individuals with Allergic Asthma**

**C. Solomon**

**J. Balmes**

*University of California, San Francisco*

**M. Kleinman**

*University of California, Irvine*

# Introduction

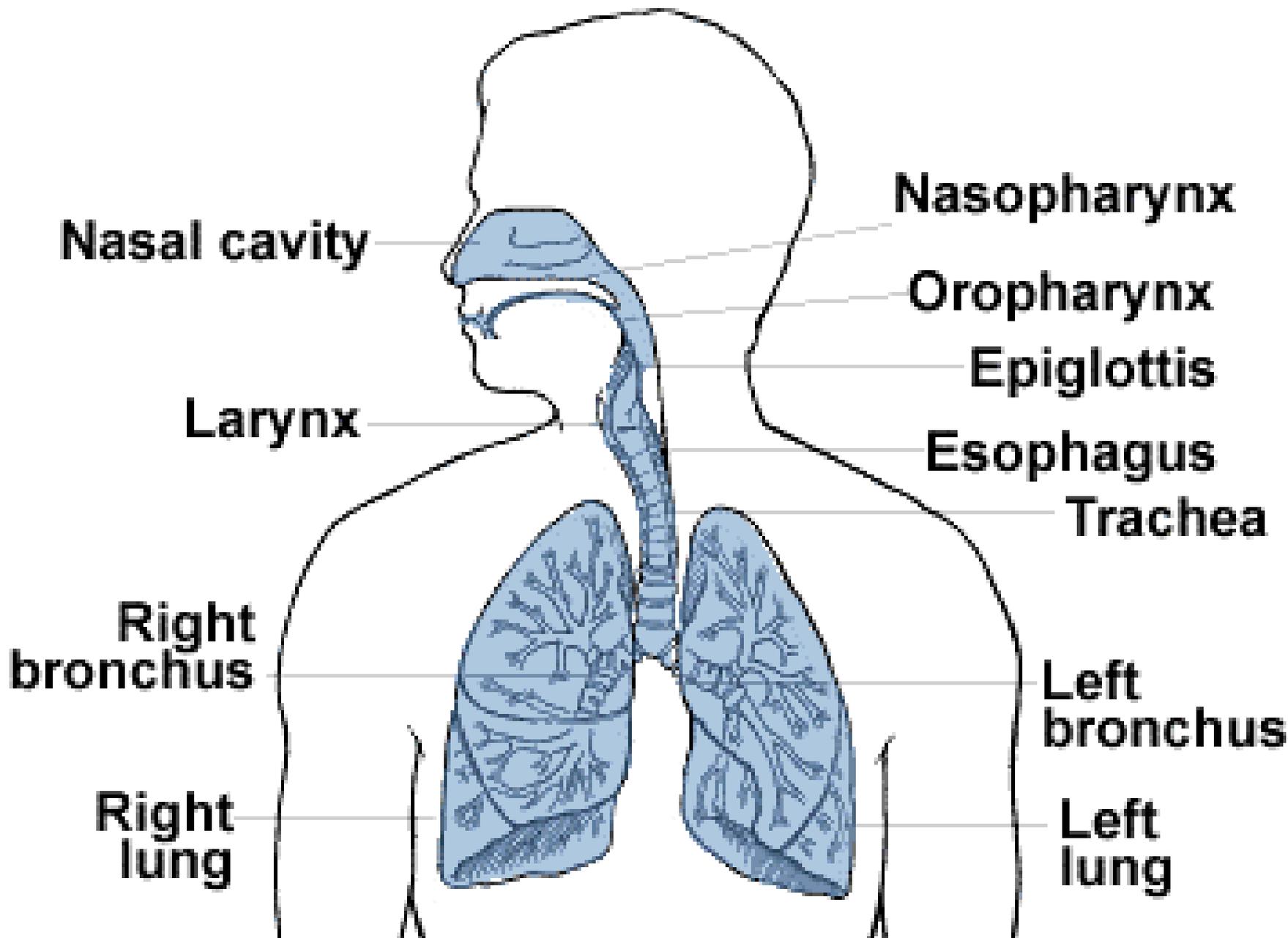
- Nitrogen dioxide ( $\text{NO}_2$ ) is a major air pollutant world-wide
- Environmental sources
  - combustion of fossil fuels
    - motor vehicle exhaust
    - stationary sources (industrial, heating, power generation)
- Residential
  - gas and kerosene appliances
- Occupational
  - welding

# Introduction

- **Outdoor concentrations:**
  - **California; up to 0.27-0.31 ppm (1 hr average)**
- **Indoor concentrations:**
  - **0.12-1.09 ppm (1 hr average); peaks up to 2-4 ppm**
- **California air quality standard: 0.25 ppm for 1 hr**
- **Precursor in the formation of tropospheric ozone via photochemical reactions**

# **NO<sub>2</sub> Deposition**

- **Up to 90% of inhaled NO<sub>2</sub> absorbed in the airways**
- **Deposited throughout the respiratory tract**
- **Maximum at the junction of conducting and respiratory airways**
- **Increases with exercise**



# **NO<sub>2</sub> Toxicity**

- **Oxidant**
- **Partly water soluble, reacts with airway lining fluid to form nitric and nitrous acids**
- **Damage via lipid pre-oxidation, cell membranes, increase in cell permeability, bronchial epithelium, decrease in ciliary beat frequency**
- **Causes cough, throat irritation, dyspnea (15-25 ppm); pneumonia, bronchiolitis (25-100 ppm); fatal pulmonary edema (>150 ppm)**

# **NO<sub>2</sub> Health Effects: Asthma**

- **Association between NO<sub>2</sub> exposure and increased respiratory symptoms, emergency department visits or hospitalization for asthma**
- **Asthmatics could be more susceptible due to:**
  - **Airway inflammation and hyper-reactivity**
  - **Allergy**

# **NO<sub>2</sub> Controlled Exposure: Asthma**

- **n = 12; 1.0 ppm NO<sub>2</sub>; 3 hr; bronchoalveolar lavage 1 hr post-exposure; no changes in cell distribution; changes in prostaglandins; decreased FEV<sub>1</sub>**
- **n = 8; 0.3 ppm NO<sub>2</sub>; 1 hr; sputum-induction 2 hr post-exposure; no change in cell distribution; no change in pulmonary function**

# Hypothesis

**It was hypothesized that exposure to 0.4 ppm NO<sub>2</sub>, compared to filter air, would induce non-specific airway inflammation, 6 hr and 26 hr post-exposure, in individuals with asthma**

# Methods

## *Design*

**Controlled human exposure experiment: single-blind, counter-balanced, repeated-measures**

## *Subjects*

- **Twelve atopic subjects with mild asthma:**
  - **7 females, 5 males**
  - **age =  $35.7 \pm 9.4$  yr**
  - **FEV<sub>1</sub> =  $75.1 \pm 9.1$  % pred**
  - **MCh PC<sub>20</sub> =  $2.0 \pm 2.3$  mg ml**

# Methods

## *Procedure*

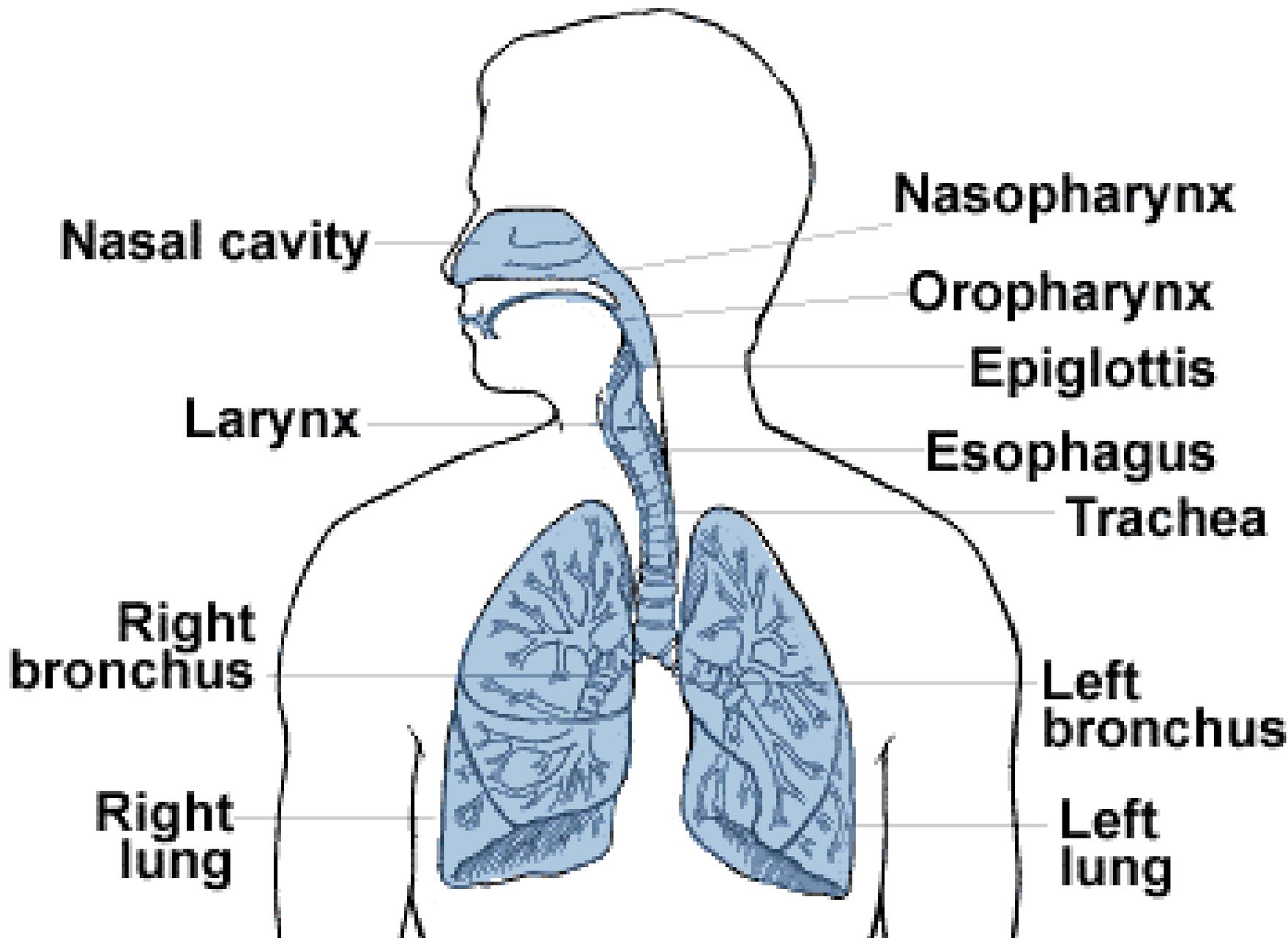
- **Exposure: Filtered Air; 0.4 ppm NO<sub>2</sub> (0.4 ± 0.017) ppm (compressed gas); chamber, 3 hr, 3 x 30 min exercise**
- **Spirometry: pre- and post-exposure, and pre-S-6**
- **Sputum induction: 6 hr and 26 hr post-exposure**
- **Cells: total and differential counts**
- **Proteins: total protein, IL-6, IL-8, GMCSF**

## *Statistical Analysis*

- **Wilcoxon Signed-Rank Test, significance set at p<0.05**

# Sputum-Induction

- **Non-invasive, safe, inexpensive**
- **Nebulized 3% saline**
- **Inhaled via mouth-piece**
- **t = 20 min; 2 min interval collection**
- **Collect sputum and saliva separately**
- **Sputum homogenized**
- **Cell counts**
- **Proteins**
- **Sample derived from central airways**
- **Controlled human exposures: NO<sub>2</sub>, ozone, particles, diesel exhaust**



Nasal cavity

Larynx

Right  
bronchus

Right  
lung

Nasopharynx

Oropharynx

Epiglottis

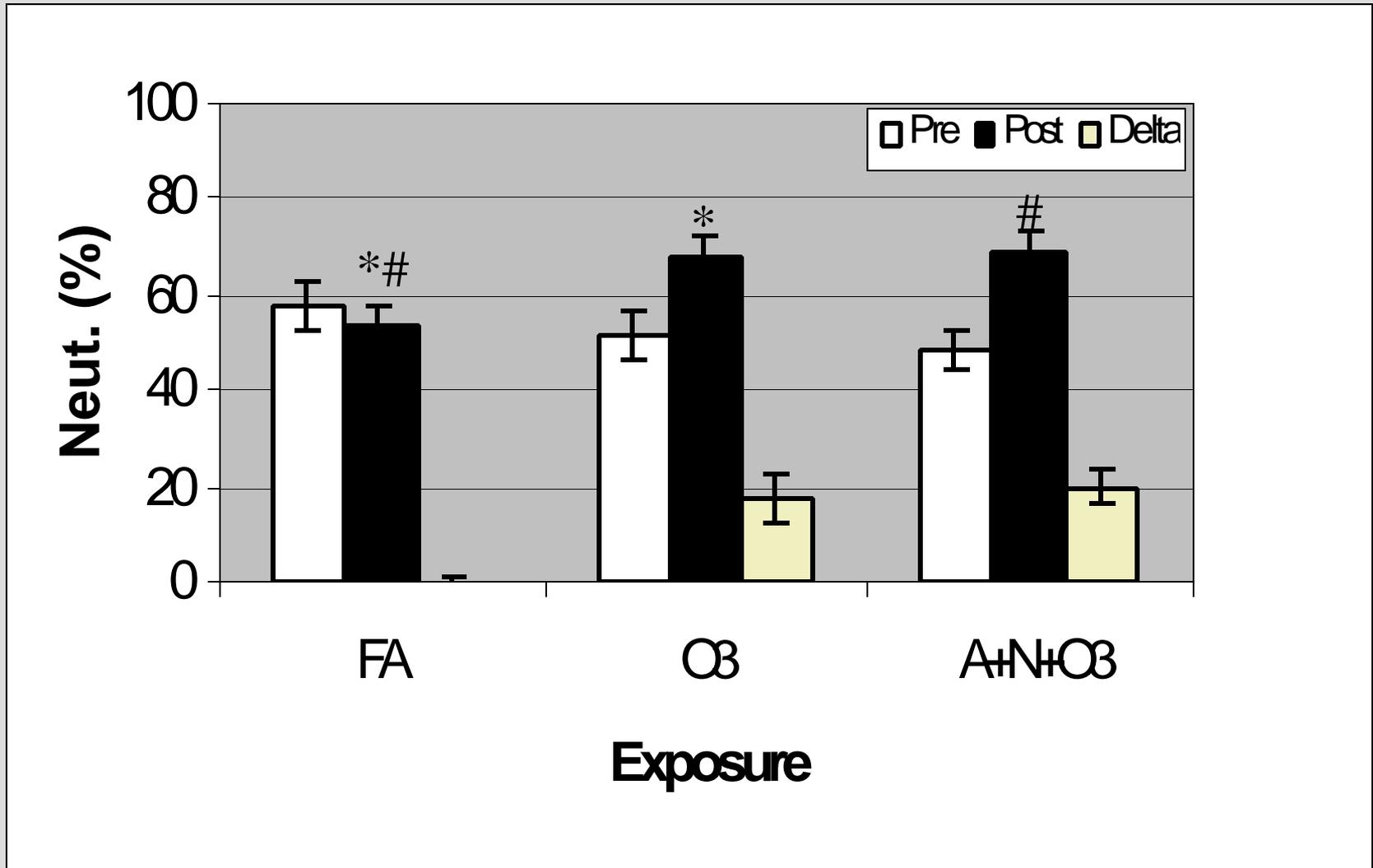
Esophagus

Trachea

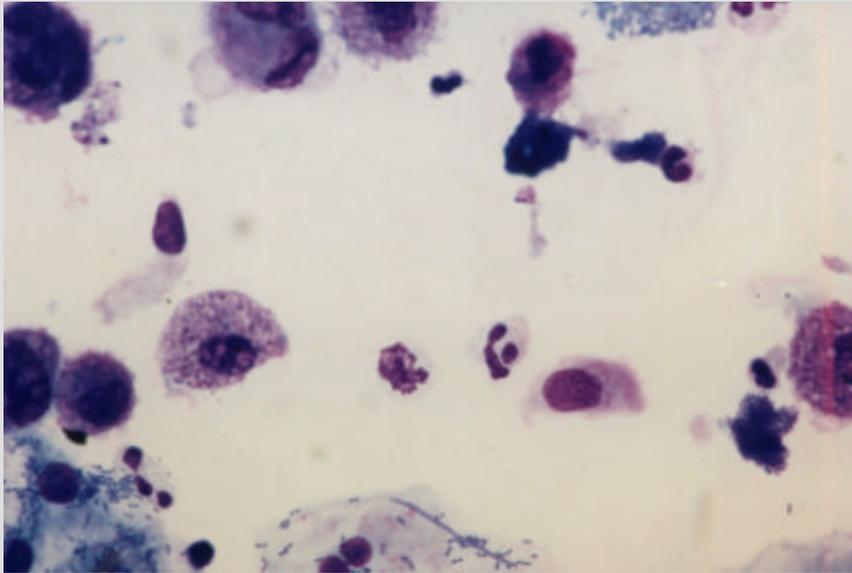
Left  
bronchus

Left  
lung

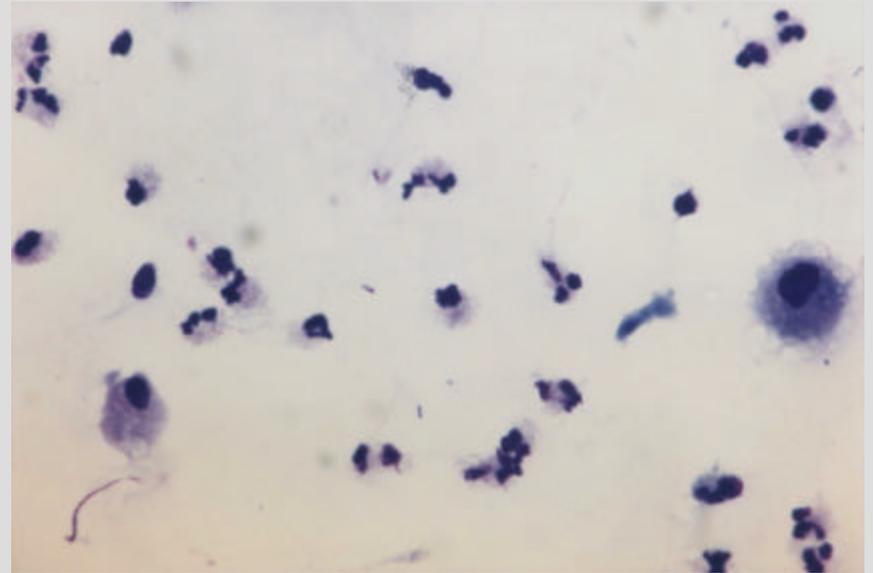
# S-I Neutrophils: Ozone, Particles, Acid



# S-I Differential Cells: Zinc-Oxide

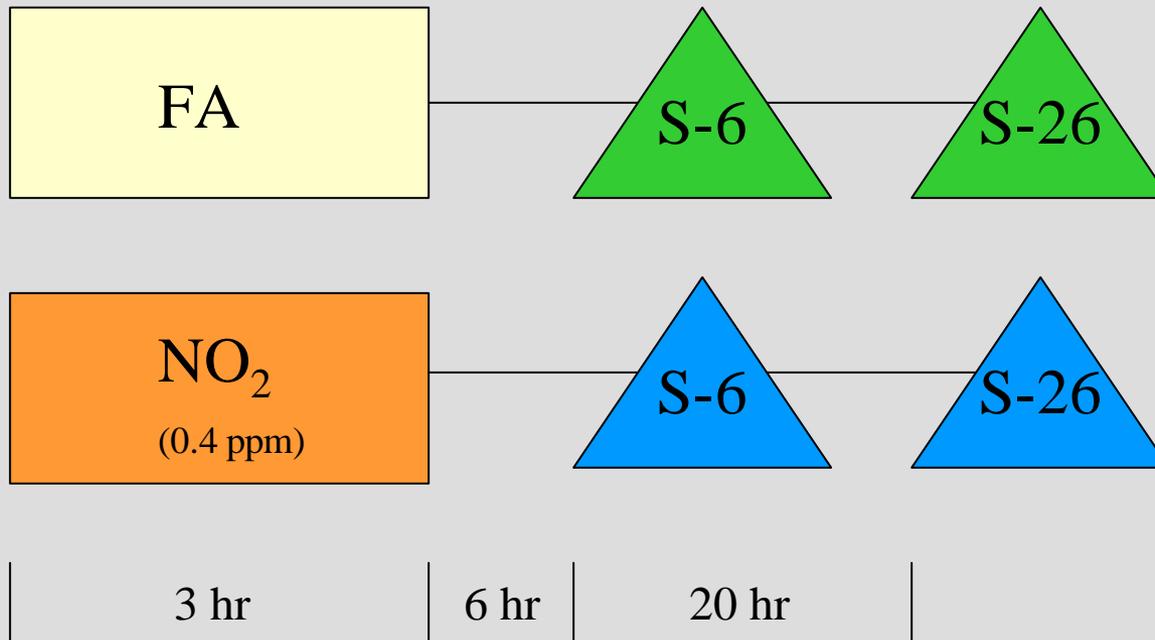


**Filtered Air**



**Zinc-Oxide**

# Study Design



*Abbreviations: FA = filtered air; NO<sub>2</sub> = nitrogen dioxide; S-6 = sputum induction at 6 hr after exposure; S-26 = sputum induction at 26 hr after exposure*

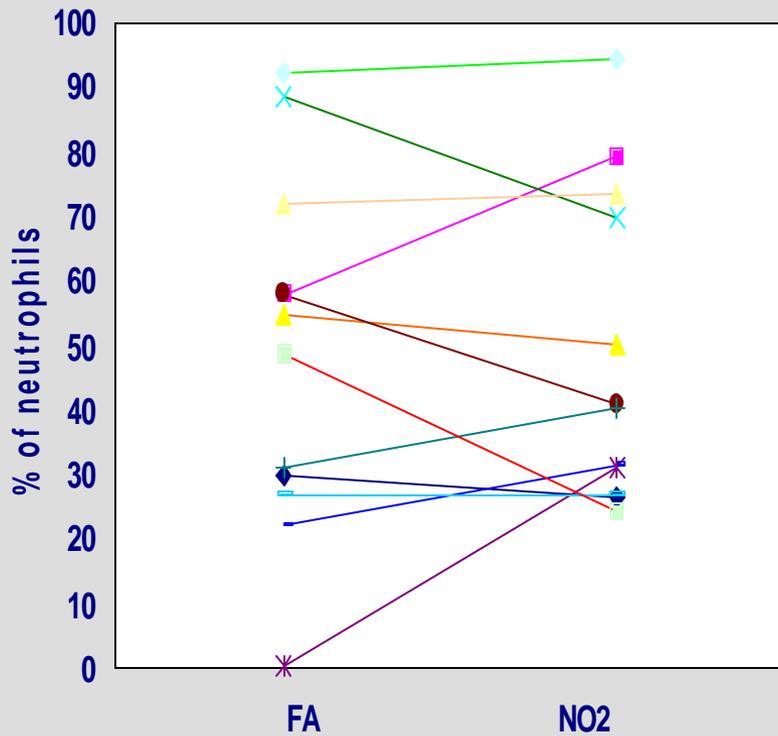
## Leukocytes in Induced Sputum at 6 hr and 26 hr Post-Exposure to Filtered Air and Nitrogen Dioxide

	S-6		S-26	
	FA	NO <sub>2</sub>	FA	NO <sub>2</sub>
Total leukocytes cells x 10 <sup>4</sup> ml	59.5 41.5 – 119.3	82.8 50.5 – 122.5	57.5 44.0 – 88.0	58.5 51.0 – 66.0
% Macrophage	35.9 14.6 – 64.8	43.2 18.5 – 53.4	38.1 29.6 – 56.1	44.1 34.3 – 46.9
% Neutrophil	59.6 30.9 – 79.9	49.9 38.1 – 79.9	56.1 41.1 – 66.2	53.1 46.3 – 62.1
% Eosinophil	1.2 0.3 – 1.8	0.8 0.5 – 3.0	1.4 0.6 – 1.8	0.5 0.0 – 4.6
% Lymphocyte	0.25 0.0 – 3.1	0.4 0.2 - 1.2	0.6 0.3 – 1.8	0.3 0.0 – 3.0
Macrophage cells x 10 <sup>4</sup> ml	28.6 7.7 – 57.6	27.9 14.6 – 31.5	21.9 15.0 – 39.8	25.1 9.5 – 29.1
Neutrophil cells x 10 <sup>4</sup> ml	41.0 18.3 – 76.2	48.8 19.6 – 91.3	34.3 18.1 – 76.6	32.6 23.6 – 53.5
Eosinophil cells x 10 <sup>4</sup> ml	0.9 0.3 – 2.1	0.9 0.5 – 2.6	0.8 0.4 – 1.3	0.3 0.0 – 2.2
Lymphocyte cells x 10 <sup>4</sup> ml	0.2 0.0 – 6.2	0.3 0.05 - 1.1	0.3 0.1 – 0.6	0.2 0.0 – 1.5

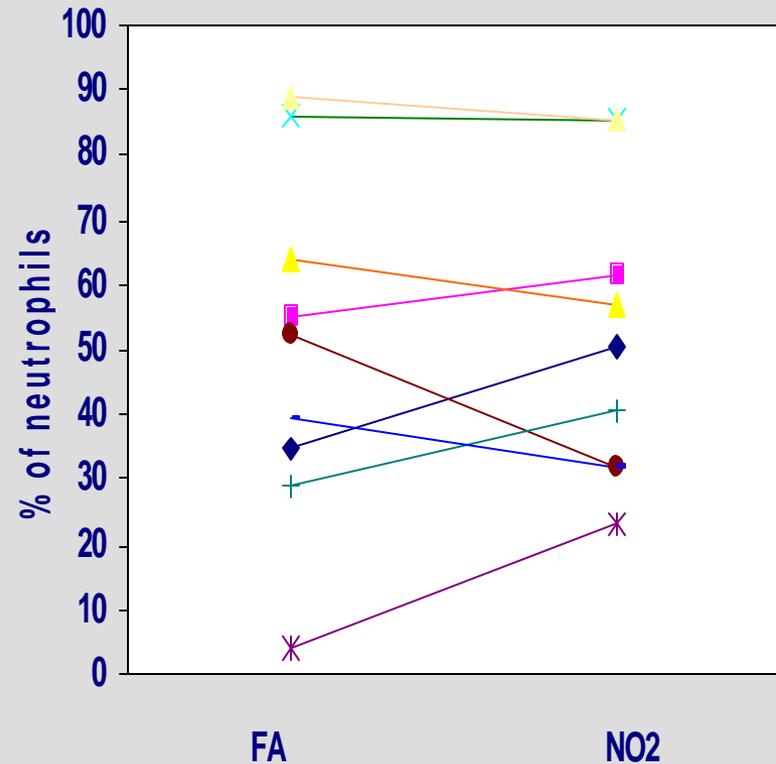
*FA: filtered air, NO<sub>2</sub>: nitrogen dioxide, S-6 and S-26: sputum induction 6 and 26 hours after exposure. Values expressed as median; interquartile range*

# Individual Differential Neutrophil Count in Induced Sputum at 6 hr (A) and 26 hr (B) Post-Exposure to Filtered Air and Nitrogen Dioxide

## A



## B



## Protein Analysis of Induced Sputum at 6 hr and 26 hr Post-Exposure to Filtered Air and Nitrogen Dioxide

	S-6		S-26	
	FA	NO <sub>2</sub>	FA	NO <sub>2</sub>
IL-6 (pg ml)	106.0 82.6 – 251.6	78.1 31.6 – 98.8	64.3 28.4 – 129.2	66.1 31.1 – 171.1
IL-8 (pg ml)	2329.7 1705.0 – 5822.4	3045.1 2289.0 – 5250.0	3347.5 1774.0 – 5951.6	2349.4 1289.5 – 4014.3
GM-CSF (pg ml)	2.2 0.9-3.2	1.1 0.6-1.5	1.1 1.0-2.3	1.8 0.2-2.5
Total Protein (mg ml)	1.62 1.60 – 1.63	2.0 1.42 – 2.88	1.67 1.66 – 2.10	2.20 1.44 – 2.75

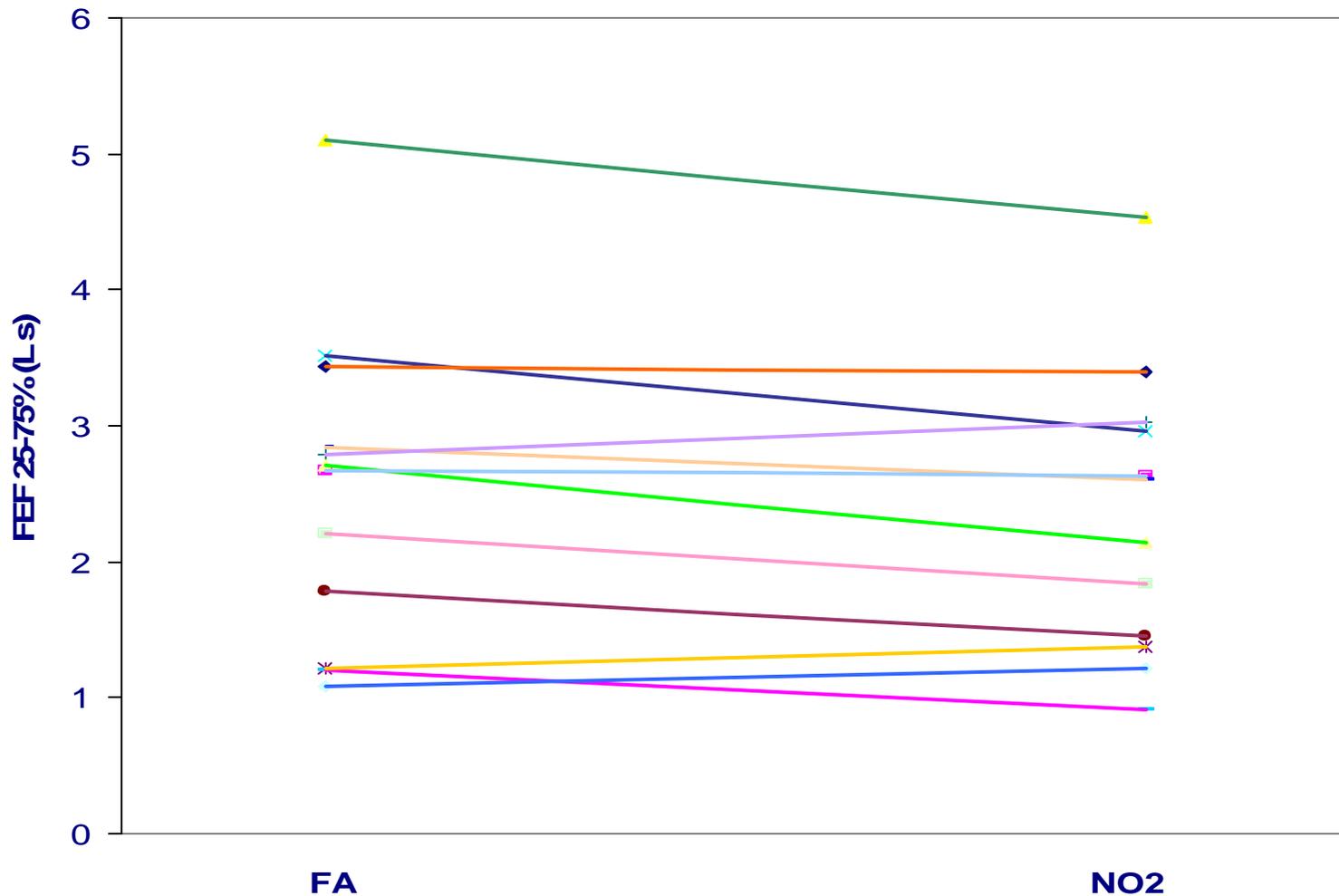
*IL-6: interleukin-6, IL-8: interleukin-8, TP: total protein. Values expressed as median and interquartile range*

## Spirometric Pulmonary Function Responses to Filtered Air and Nitrogen Dioxide

SPF	Pre-Exposure		Post-Exposure		Pre-S-6	
	FA	NO <sub>2</sub>	FA	NO <sub>2</sub>	FA	NO <sub>2</sub>
FVC (L)	3.61 3.0-4.3	3.66 3.2-4.2	3.86 3.0-4.3	3.79 2.9-4.2	3.85 2.8-4.6	3.79 2.9-4.2
FEV <sub>1</sub> (L)	2.97 2.2-3.3	2.79 2.4-3.4	2.92 2.3-3.4	2.94 2.2-3.4	2.91 2.3-3.4	2.83 2.1-3.5
FEF <sub>25-75</sub> (L s)	2.39 1.5-3.1	2.56 1.8-2.9	2.61 1.8-3.3	2.46 1.8-3.2	2.69* 1.5-3.1	2.37* 1.4-2.9

*SPF: spirometric pulmonary function, FA: filtered air, NO<sub>2</sub>: nitrogen dioxide, S-6: sputum induction 6 hr post-exposure, FVC: Forced vital capacity, FEV<sub>1</sub>: Forced expiratory volume in one second, FEF<sub>25-75</sub>: Mean forced expiratory flow rate between 25-75% of FVC, \* = significantly different (p=0.04). Values as median; interquartile range*

# Individual FEF<sub>25-75%</sub> Values at 6 hr Post-Exposure to Filtered Air and Nitrogen Dioxide



# Discussion

- The results of this project indicate that in individuals with asthma, exposure to 0.4 ppm NO<sub>2</sub> results in:
  - Decrease in spirometric pulmonary function
  - No changes in inflammation-associated cells
  - No changes in cytokine proteins

# Discussion

- Maximal NO<sub>2</sub> deposition and S-I sampling region
- Sputum-induction variability:
  - With-in subjects
  - Between subjects
  - Asthma: disease status

# Discussion

- **Bronchoscopy**
- **Decrease in  $FEF_{25-75\%}$  potentially in the region of higher  $NO_2$  deposition**
- **Individual susceptibility**

# Acknowledgements

## UCSF

Chandreyi Basu

Allyson Witten

## UC Irvine

Michael Kleinman

## Funding

California Air Resources Board