# $NO_2$ – evidence of direct health effects from toxicological studies?

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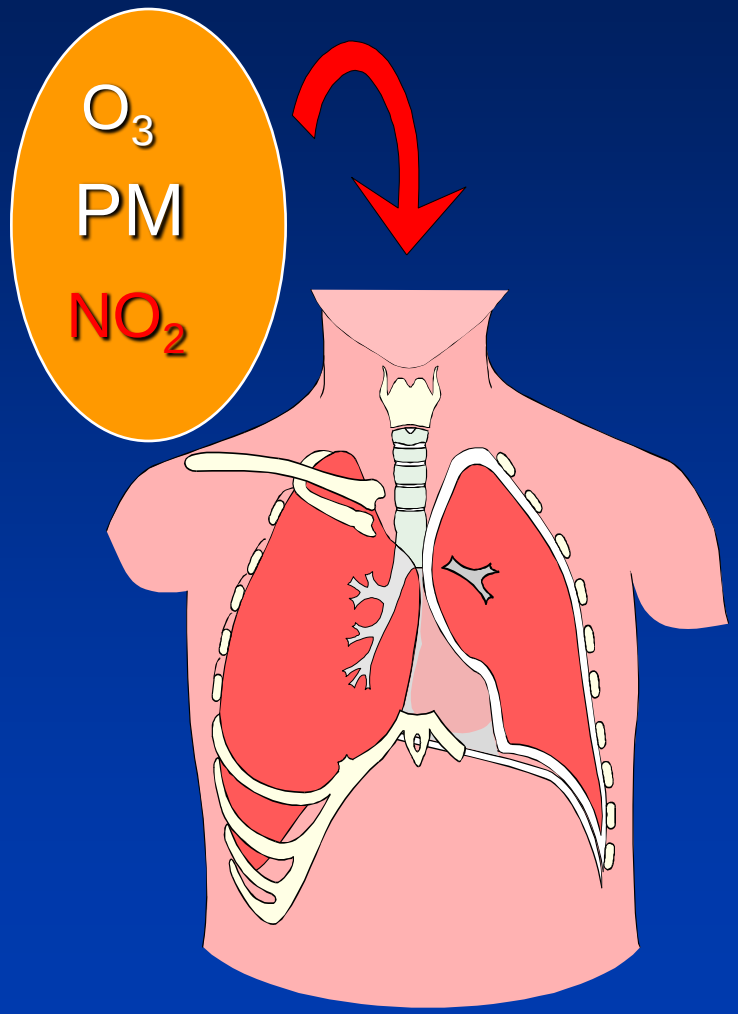
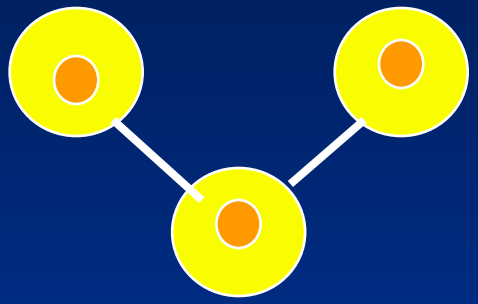
# Nitrogen dioxide

Free radical – very reactive

# ‘Oxidant’ or free radical theory of air pollution




Powerful oxidant



Surface components drive oxidative reactions

Free radical

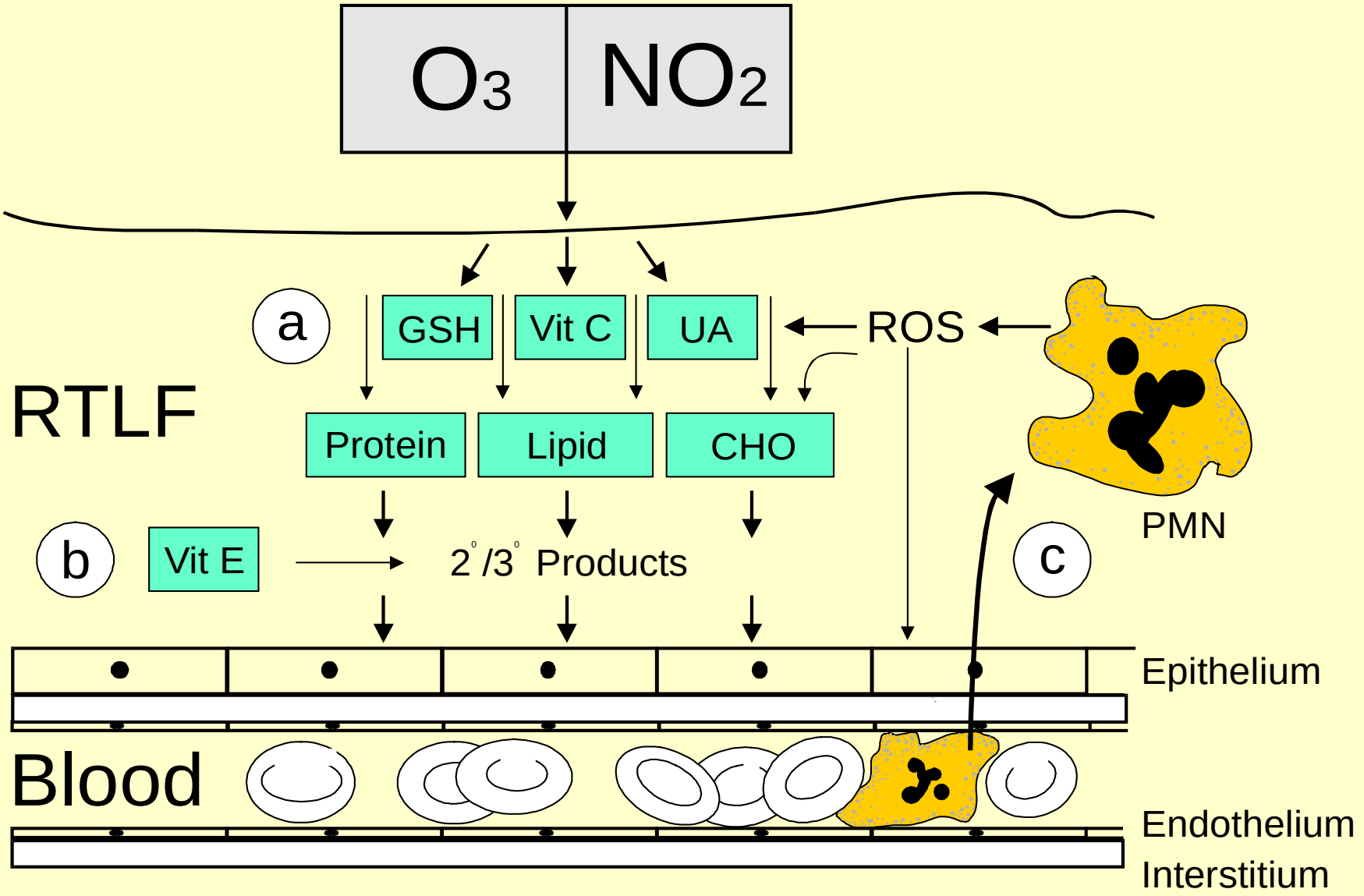
## What do free radicals do?

- deplete antioxidants
- cause damaging oxidation reactions (oxidise proteins, lipids and DNA)

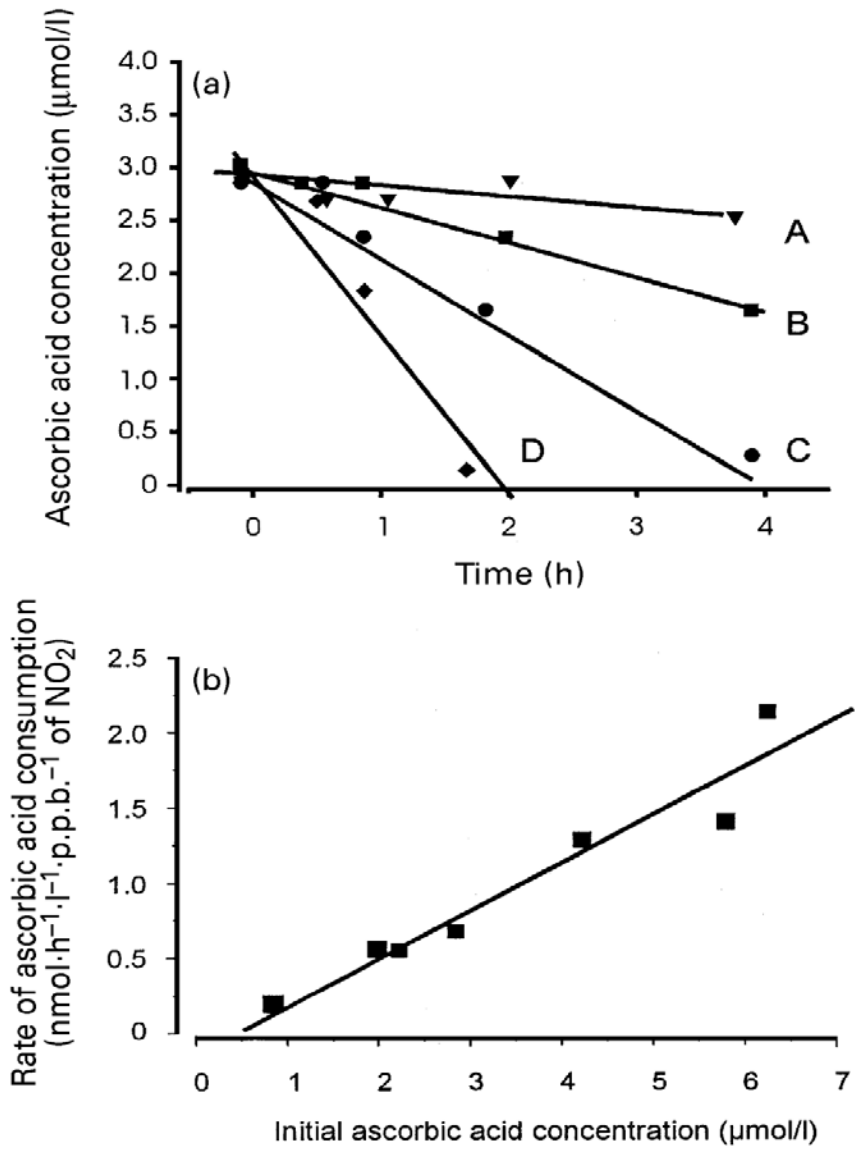
## Why is this important?

- Altered redox status triggers inflammation
- Oxidation of proteins will alter their function
- Oxidation of lipids will damage cell membranes – disrupt integrity/tissue injury

# Oxidant Gas Interactions at the Surface of the Lung



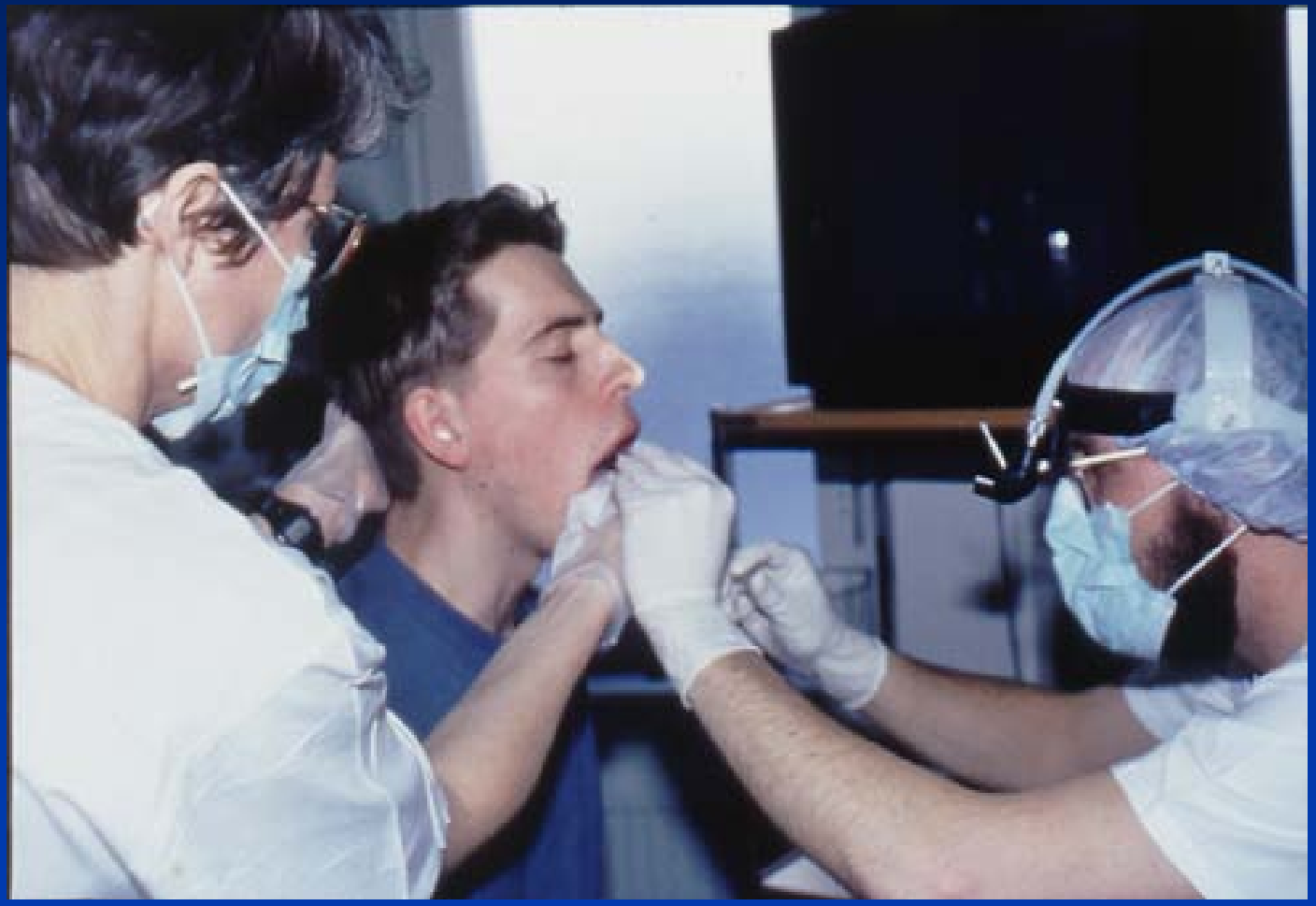
# BAL fluid AA consumption by NO2



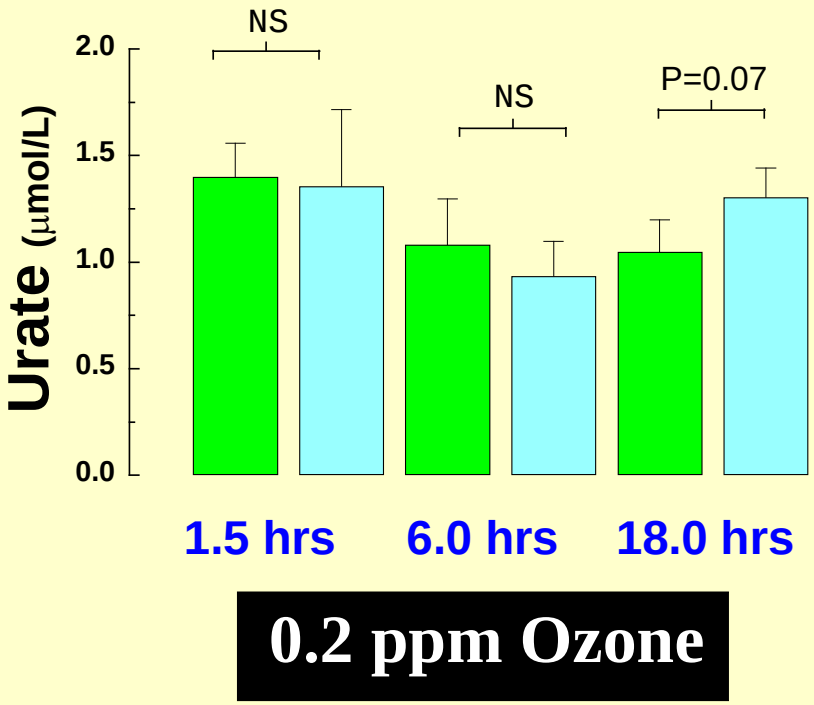

**Kelly & Tetley, Biochemical Journal (1997) 325, 95-99**

# Human exposure facility at Umea University, Sweden

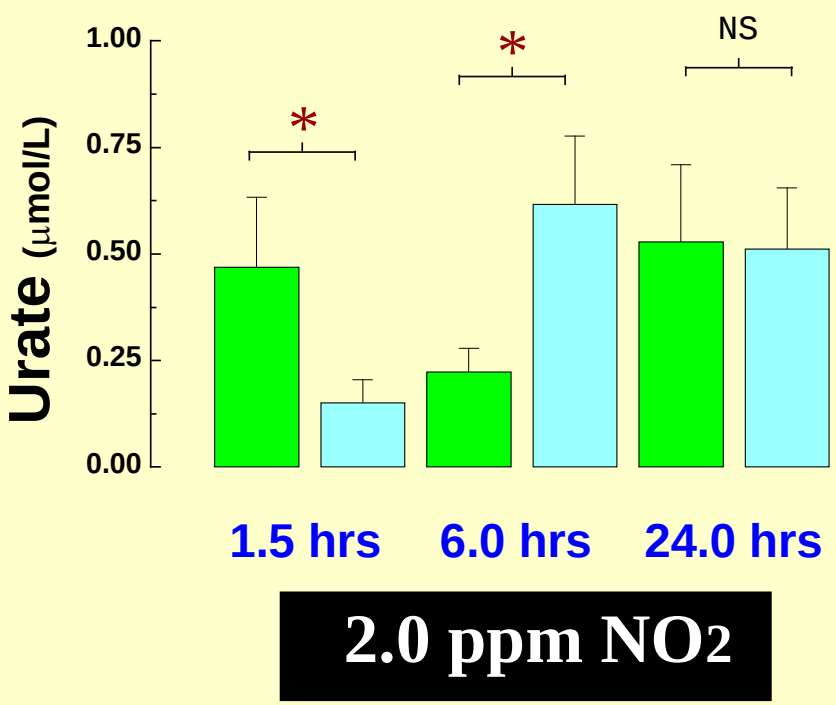
# Bronchoscopy with bronchoalveolar lavage



# RTLF Urate Responses to $O_3$ and $NO_2$



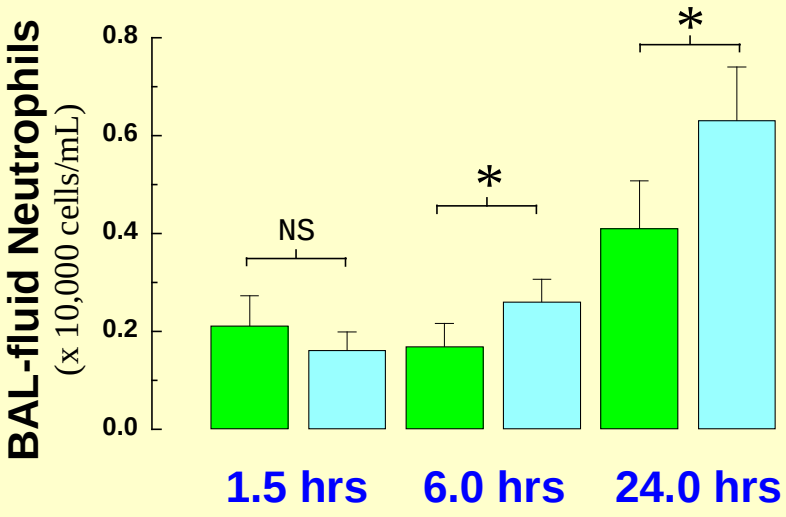

**0.2 ppm Ozone**




**2.0 ppm $NO_2$**

Filtered air  Oxidant gas

# RTLF Neutrophil Responses to $O_3$ and $NO_2$

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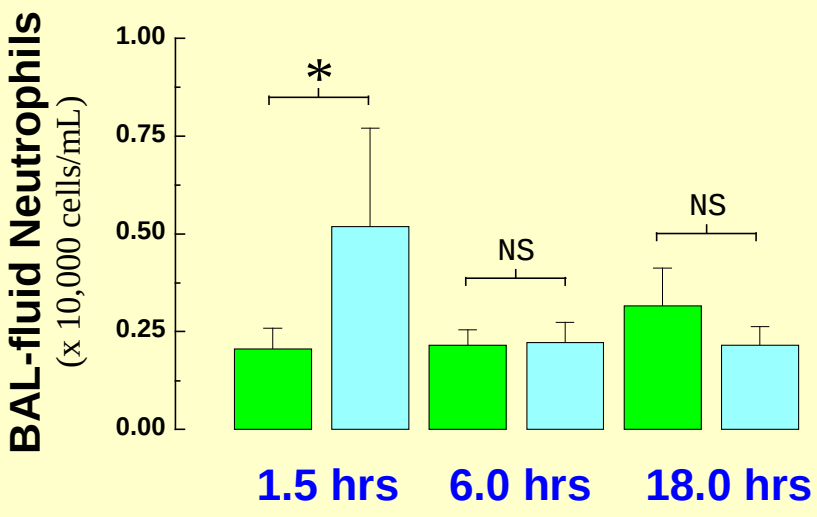


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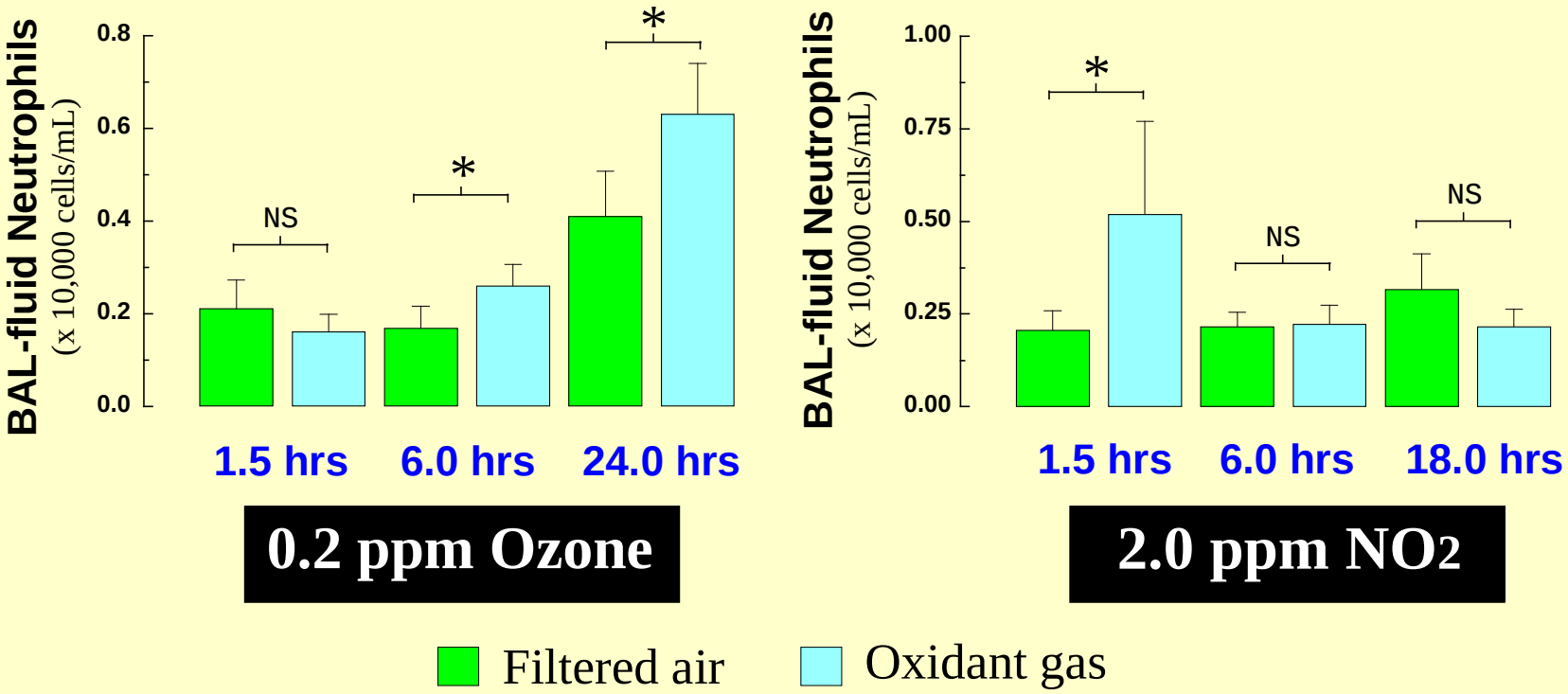
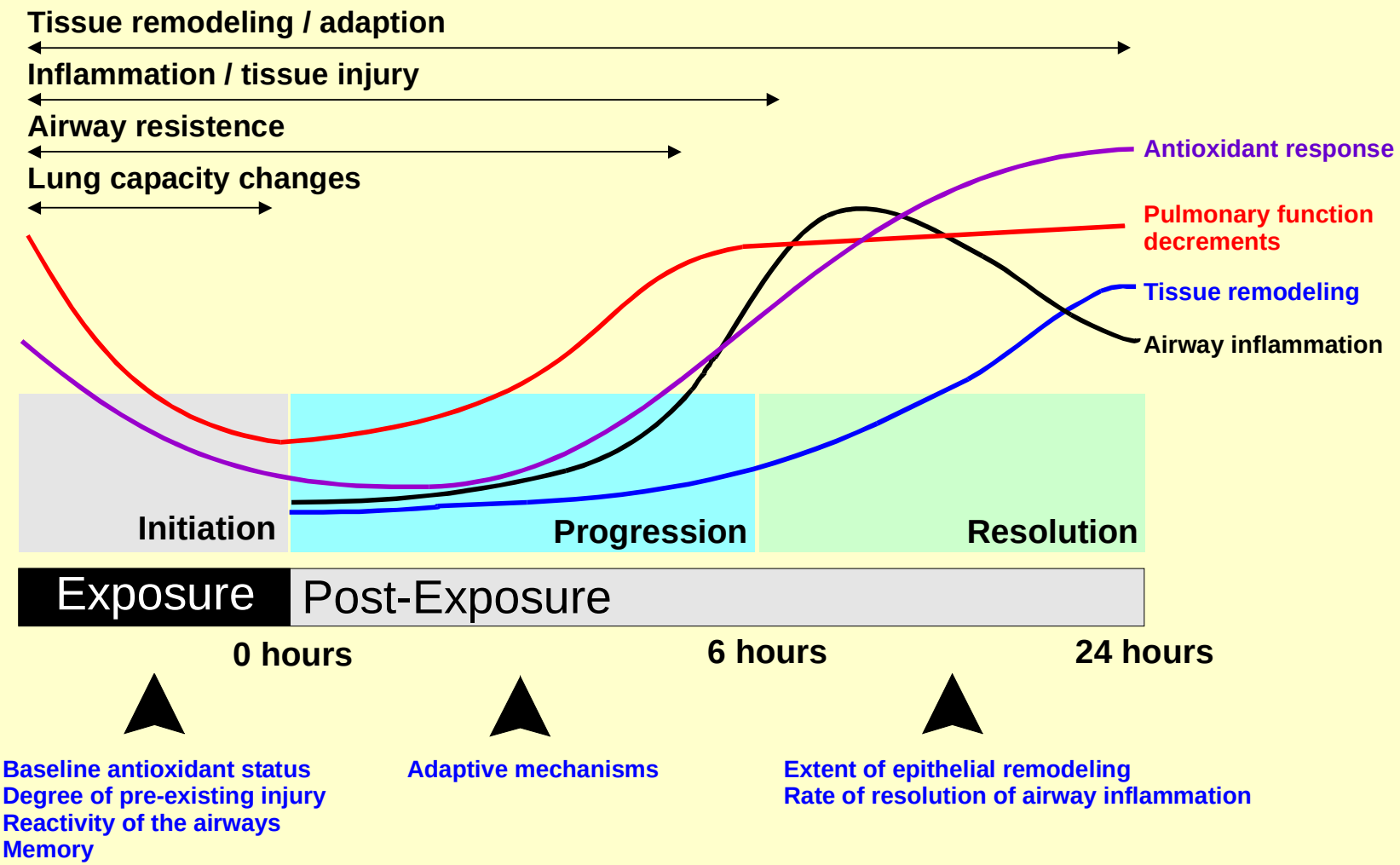



- No evidence of neutrophil activation (MPO) at any time point

# Acute Responses of the Airways to Oxidant Gases



# $NO_2$ and health effects

**Evidence from …**

- In vitro studies
- Animal toxicology
- Controlled human exposures

# $NO_2$ and health effects

**Evidence from …**

- Animal toxicology
  - Pulmonary metabolism
  - Pulmonary structure
  - Pulmonary function
  - Airway inflammation/responsiveness
  - Host defences

# $NO_2$ animal toxicology
## - pulmonary metabolism -

| | | |
|---|---|---|
| • Lung oedema<br>• Lipid changes<br>• ↑antioxidant metabolism<br>• ↑lung enzymes | >3160 µg/$m^3$<br>(acute & subchronic) | Rats |
| • ↑ lipid peroxidation | 752 µg/$m^3$<br>(18 mo; TBARS)<br>75 µg/$m^3$<br>(9 mo; ethane exhalation) | Rats |

- *Lipid & antioxidant metabolism show response pattern dependent on conc. & exposure duration*

# $NO_2$ animal toxicology
## - pulmonary structure -

| | | |
|---|---|---|
| Cell changes (type I alveolar epithelial to type II; ciliated epithelial to non-ciliated) in tracheobronchial & alveolar regions | 640 µg/$m^3$ (?) | Rats |
| Cytoplasm changes & hypertrophy in replaced cells | 940 µg/$m^3$ (10 d) | Rats |
| Human-type emphysema | 15000-37000 µg/$m^3$ (chronic) | Rats/ rabbits |

- *Both conc. & time of exposure important, but pattern is complex*

# $NO_2$ animal toxicology
## - pulmonary function -

| | | |
|---|---|---|
| ↑ Breathing frequency<br>↓ distensibility and gas exchange | 1880-9400 µg/$m^3$ (acute?/sub-chronic): | Rats |
| ↓ Thoracic clearance | 18000 µg/$m^3$ (chronic) | Ferrets |

# $NO_2$ animal toxicology
## airway inflammation/responsiveness

| | | |
|---|---|---|
| ↑ epithelial damage, baseline smooth muscle tone & airway neutrophilia; ↓ mucin expression | 3760 µg/m$^3$ (24 h) aerosolised OVA on d13 & 14 | BALB/c mice sensitised to OVA |
| ↓ TNF-α; ↑ IL-10, IL-6 & suppressor of cytokine signalling-3 mRNA | 18800 µg/m$^3$ (1,3,20 d) | Rats |

- *In vitro, depleted antioxidants defences, cell injury & inflammation confirm reactivity of $NO_2$*

# $NO_2$ animal toxicology
## - host defence -

| | | |
|---|---|---|
| ↓ Antibacterial defence | 940 µg/m$^3$ - 6 mo<br>3760 µg/m$^3$ - 3 h | Mice |

- *Effects due more to concentration than duration or total dose*
- *Peak exposures and patterns of exposures important*

# Animal toxicology
## - summary -

- Exposure  to above ambient concentrations: effects on lung metabolism, structure, function, inflammation & increased susceptibility to infection
- Very high concentrations: emphysema-like changes

# $NO_2$ animal toxicology
## - extrapolation to humans -

Inherent differences between mammalian species

Is $NO_2$ an inhalant toxicant at ambient concentrations in humans?

Exactly what exposures would lead to these effects in humans?

Would some effects seen in animals occur in humans at all?

# $NO_2$ and health effects

**Evidence from …**

- In vitro studies
- Animal toxicology
- Controlled human exposures

# $NO_2$ and health effects

**Evidence from …**

- Controlled human exposures
  - Pulmonary function
  - Airway responsiveness in asthmatics
  - Airway inflammation
  - Host defence

# $NO_2$ controlled human studies
## - pulmonary function -

| | | |
|---|---|---|
| Healthy subjects | >1800 µg/m$^3$<br>9400 µg/m$^3$ but not at 7000 µg/m$^3$<br>2820 – 6580 µg/m$^3$ (20') | *Generally*<br>↑ $SR_{aw}$<br>↓Mucociliary Cl |
| Asthmatics | 230 & 188 µg/m$^3$ (?)<br>560 µg/m$^3$ (30-110' + exercise)<br>1880-7520 µg/m$^3$ (?) | ns trends<br>Lowest level<br>No response |
| COPD | 560 µg/m$^3$ (4h)<br>Similar to above (1h + exercise)<br>3000 µg/m$^3$ (?) | Functional effects<br>No response<br>↑ $SR_{aw}$ |

# $NO_2$ controlled human studies
## - airway responsiveness in asthmatics -

| | |
|---|---|
| 560 µg/m$^3$<br>488 µg/m$^3$ | Cold<br>Histamine |
| meta-analysis<br>≥ 200 µg/m$^3$<br>≥ 1900 µg/m$^3$ (normals) | Increase in airway responsiveness to a range of constrictor stimuli |
| 800 µg/m$^3$<br>500 µg/m$^3$ | House-dust mite allergen<br>Pollen allergen |
| ≥ 300 µg/m$^3$ ('road tunnel $NO_2$') | Greater early response; ↓ function and ↑ symptoms during late response |

*Mechanistic studies: ↑ neutrophils in BW & BAL; ↑ECP in BW, blood & sputum; ↑eosinophil granule product in BW*

# $NO_2$ controlled human studies

## - airway inflammation -

| | |
|---|---|
| <u>Single dose</u><br><br>Healthy subjects<br>1128-7520 µg/$m^3$ | ↑ neutrophils, IL-8, antiprotease, $\alpha_2$-macroglobulin<br>↓/↑ mast cells & lymphocytes<br>↓ alveolar macrophages & $\alpha_1$-protease inhibitor activity |
| <u>Repeated dose</u><br><br>Healthy subjects<br>3600 µg/$m^3$ 4h/d x4 | ↑ neutrophils<br>↓ antioxidants<br>Upregulation in expression of IL-5, IL-10, IL-13 & ICAM-1 |

# $NO_2$ controlled human studies
## - host defence -

| | |
|---|---|
| <u>Healthy subjects</u><br><br>1880-5600 µg/$m^3$ 2h/d x3<br>Attenuated influenza virus | ns trend for increased infectivity |
| <u>Healthy subjects</u><br><br>1128 µg/$m^3$ 3h<br>Attenuated influenza virus | ↓ inactivation of virus by alveolar macrophages |

# $NO_2$ controlled human studies
## - interaction with other pollutants -

| | |
|---|---|
| Healthy subjects<br>1130 µg/m$^3$ + $O_3$ | ↑ responsiveness to methacholine |
| Asthmatics<br>720 µg/m$^3$ + 7000 µg/m$^3$ $SO_2$<br>752 µg/m$^3$ + 428 µg/m$^3$ $O_3$ | ↑ airway response to allergen |
| Healthy subjects<br>1000 µg/m$^3$ + 100 µg/m$^3$ $PM_{10}$<br>2700 µg/m$^3$ + 300 µg/m$^3$ $PM_{10}$ | ↑ oxidative stress; neutrophil, mast cell & lymphocyte infiltration; ↑ adhesion molecule expression; activation of bronchial epithelium → multitude of inflammatory cytokines |
| Elderly +/- COPD<br>752 µg/m$^3$ + µg/m$^3$ $PM_{2.5}$ | No significant response attributable to separate or combined effects |

# $NO_2$ controlled human studies
## - summary -

- In healthy subjects, changes in pulmonary function, ↑ airway responsiveness, mild inflammation & ↓ host defences at concentrations (>1800 µg/$m^3$ +/- co-pollutant) in excess to those outdoors
- Asthmatics more susceptible to acute effects
- In mild asthmatics, lowest concentration to change pulmonary function: 500 µg/$m^3$ and to enhance effect of allergens: 200 µg/$m^3$

# - $NO_2$ guidelines -

What are the values protecting us from?

**$NO_2$**

- Precursor of secondary toxic pollutants
- Marker of traffic-related pollution
- Potential to enhance other environmental pollutants eg allergens

Guideline that limits resulting health effects

Reductions in $NO_2$ *PLUS* secondary traffic related pollution +/or secondary pollutants

# $NO_2$ - a surrogate for traffic or a pollutant in its own right?

## Questions to be addressed:

- Does $NO_2$ at concentrations achieved outdoors have any detectable toxicity on the human lung ?
- Which aspects or components of combustion mixtures are responsible for the adverse health effects observed in epidemiological studies ?
- Is $NO_2$ able to synergise with other pollutants eg PM (ie role as an effect modifier) ?

More efficient protection against health effects of complex gas-particle mixtures ?

# - $NO_2$ annual guideline -

- Set to protect the public from health effects of $NO_2$ itself
- Still no robust basis for setting a value for $NO_2$ through any direct toxic effect
  - Increased concern over health effects from recent epidemiological studies
  - Possible contribution from unmeasured components (eg organic carbon, nitrous acid vapour)
- Takes into account a potential direct toxic effect of chronic $NO_2$ exposure at low levels