Frontiers in Air Quality
Advances in Air Quality Toxicology

Heart and Lungs – Victims of Polluted Air

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How did it start?

THE LONDON SMOG

CONCENTRATION OF SMOKE, SULFUR DIOXIDE, mg/m$^3$

DATE IN DECEMBER 1952

Deaths

Sulfur dioxide

Smoke

FOG

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

DEATHS PER DAY

0 250 500 750 1000
Combustion-derived particulate matter air pollution

- Diesel exhaust
  - Petro diesel
  - Biodiesel
- Wood smoke/biomass burning
Different air pollution sources – different sizes

Coarse <10 µm
Fine <2.5 µm
Ultrafine <0.1 µm
Particle sizes
Petro diesel vs. Biodiesel RME100

Petro diesel

Biodiesel

![Graph comparing Particle mobility diameter (nm) for Petro diesel and Biodiesel RME100](image)

![Graph comparing Number concentration (#/cm^3) for Petro diesel and Biodiesel RME100](image)
Human exposure chamber studies

- Selected populations
  - Healthy, allergy, asthma, COPD, elderly
- Exposure situation mimicking real life
  - Traffic situations, work places
- Controlled exposure concentrations
- Predetermined workload/ventilation rate
- Randomised sequence
  - Filtered air ⇋ air pollutant
Exposure Setup

Diesel exhaust used as a model of PM pollution

Exposure to diesel exhaust and filtered air for 1-2 hours on two separate occasions
Bronchoscopy
Exposure to diesel exhaust in healthy volunteers - *biopsies*

Filtered air

Diesel exhaust

Neutrophils

Salvi et al AJRCCM 1999
Airway effects by diesel exposure in healthy humans

MAPKs
- p38

Transcription factors
- AP-1, NFκB

Cytokines
- IL-8, IL-13, GRO-α

Adhesion molecules
- ICAM-1, VCAM-1, LFA-1

Inflammatory cells
- Neutrophils, mast cells, lymphocytes
Diesel exhaust – PM concentrations 100 vs. 300 µg/m$^3$

- Slower development of airway inflammation at a lower concentration
- Events occurring at 6 hours after 300 µg/m$^3$ can be found 18 hours after 100 µg/m$^3$
Diesel exhaust increases airway hyperresponsiveness in asthmatics..

..despite treatment with inhaled corticosteroids

Nordenhäll et al, ERJ 2001
# Exposure to diesel exhaust

Healthy vs. Asthmatics (6 h)

<table>
<thead>
<tr>
<th></th>
<th>Healthy</th>
<th>Asthmatics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cells</strong></td>
<td>(PMN), Ly</td>
<td></td>
</tr>
<tr>
<td><strong>Cytokines</strong></td>
<td>IL-6, IL-8</td>
<td>IL-10</td>
</tr>
<tr>
<td><strong>biopsies</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Adhesion molecules</strong></td>
<td>P-selectin, VCAM-1</td>
<td></td>
</tr>
<tr>
<td><strong>Lung function</strong></td>
<td>$R_{AW}$</td>
<td>$R_{AW}$</td>
</tr>
</tbody>
</table>

Do asthmatics react more/worse at a later time point after diesel exhaust exposure or in another way?

Stenfors et al. ERJ 2004
Diesel exhaust - asthmatics

A  Submucosa neutrophil counts

B  Bronchial wash neutrophil counts

D  Bronchial wash myeloperoxidase

E  Bronchial wash Interleukin 6

Behndig A F et al. Thorax 2011
PEF-responses in asthma

Subjects with Moderate Asthma

PEF change from baseline (ml)

Time from Start of Exposure (h)
From lungs to heart......
Tools for investigating cardiovascular events of air pollution in humans \textit{in-vivo}

- Forearm plethysmography
- \textit{Ex-vivo} model of thrombosis
- Coagulation markers
- Arterial stiffness
- Blood pressure
- Heart rate and rhythm
Measuring endothelial function

VENOUS OCCLUSION PLETHYSMOGRAPHY

Non-infused

Cuff inflated

Cuff deflated

Infused arm

BILATERAL VENOUS SAMPLING
Decreased forearm blood flow 6 hours after diesel exhaust exposure

Exposure to dilute diesel exhaust for one hour impairs endothelium dependent and independent vasomotor function

Mills et al. Circulation 2005
Endogenous fibrinolysis – tissue plasminogen activator (t-PA) release at 6 hours

Area under the curve for t-PA release was reduced by 33% following diesel exhaust exposure

Mills et al. Circulation 2005
Ischemic and Thrombotic Effects of Dilute Diesel-Exhaust Inhalation in Men with Coronary Heart Disease

Nicholas L. Mills, M.D., Håkan Törnqvist, M.D., Manuel C. Gonzalez, M.D., Elen Vink, B.Sc., Simon D. Robinson, M.D., Stefan Söderberg, M.D., Ph.D., Nicholas A. Boon, M.D., Ken Donaldson, Ph.D., Thomas Sandström, M.D., Ph.D., Anders Blomberg, M.D., Ph.D., and David E. Newby, M.D., Ph.D.
Effect of exposure to diesel exhaust in patients with stable coronary heart disease

- 20 male patients
- Coronary heart disease successfully treated with PCI – stable disease
- No diabetes mellitus
- No congestive heart failure
- Normal maximal exercise test
- No symptoms
- Full “protective” medication

Exercise-induced ischaemia
Impaired endogenous fibrinolysis

Thrombus formation *ex-vivo*

Lucking *et al.*, Eur Heart J 2008
Idling v.s urban running cycle
Vascular responses similar regardless of idling or city cycle

Barath et al. Part Fibre Toxicol, 2010
PARTICULATE TRAP-STUDY
DIESEL EXHAUST vs. FILTERED DIESEL EXHAUST

Endothelium-dependent

Endothelium-independent

Lucking A et al, Circulation 2011
t-PA RELEASE

P=0.03

Bradykinin (pmol/min)

FILTERED
DIESEL
Reduced thrombus formation with filter

![Graph showing thrombus area comparison between AIR, DIESEL, and FILTERED exposures.]

- AIR: Thrombus Area (µm²)
- DIESEL: Thrombus Area (µm²)
- FILTERED: Thrombus Area (µm²)

Statistical significance:
- p=ns
- p<0.05
Vascular Effects of Diesel Exhaust – SUMMARY

- Arterial stiffness increases
- Reduced vasomotor response
- Reduced t-PA release
- Increased platelet adhesion
- Increased tendency for thrombus formation
- Mediated through the L-arginine-NO pathway
- Effects may be reduced by a particle trap
Biodiesel
RME - Rapeseed Methyl Ester

• 5-7% biodiesel included in diesel fuels in Sweden today - RME is the dominating addition
• 30 or 100% RME is used in some vehicles, due to lower price as well as being renewable
• RME may potentially be more widely used, as a 30% blend can be used without engine alternations or increased engine wear
Three exposure studies in healthy human subjects with RME Biodiesel vs. petro diesel or filtered air

1. RME30 (30% biodiesel) vs. petro diesel
   - PM$_1$ 300 µg/m$^3$ - *i.e. equal PM mass*

2. RME100 (100% biodiesel) vs. petro diesel
   - PM$_1$ 300 µg/m$^3$ petro diesel vs. 165 µg/m$^3$ RME100, *i.e. equal engine load*

3. RME100 (100% biodiesel) vs. filtered air
   - PM$_1$ 165 µg/m$^3$ RME100 vs. filtered air
   - Measurements of vascular effects 2-4 hours post exposure (studies 1-2)
   - Bronchoscopy with endobronchial biopsy and BW/BAL sampling 6 hours post exposure (study 3)
RME30 vs. petro diesel
Bilateral forearm plethysmography

Similar vascular effects by RME30 and standard petro diesel

Unosson et al, manuscript
100% RME Biodiesel vs. petro diesel
Bilateral forearm plethysmography

Similar vascular effects by RME100 and standard petro diesel

Unosson et al, manuscript
No differences in thrombus formation

Unosson et al, manuscript
100% RME Biodiesel vs. filtered air
Bronchoscopy

<table>
<thead>
<tr>
<th>Bronchial Wash (BW)</th>
<th>Filtered air (median (IQR))</th>
<th>Biodiesel (median (IQR))</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macrophages</td>
<td>4.89 (3.40-7.32)</td>
<td>8.86 (3.19-12.28)</td>
<td>0.036</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>0.93 (0.68-1.75)</td>
<td>1.80 (0.92-2.50)</td>
<td>0.008</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>0.25 (0.12-0.51)</td>
<td>0.41 (0.07-0.81)</td>
<td>0.233</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>0.00 (0.00-0.00)</td>
<td>0.01 (0.00-0.02)</td>
<td>0.314</td>
</tr>
</tbody>
</table>

Values are given as medians (IQR). Wilcoxon signed ranks test.

Airway inflammatory responses similar as seen after exposure to petro diesel exhaust. Biopsy data under progress.
Biological pathways linking PM exposure with CVDs

Blood
- PM or constituents in the circulation
  - UFP, soluble metals
  - Organic compounds
- Vasculature
  - Vasoconstriction
  - Endothelial dysfunction
  - PM-mediated ROS
  - BP
  - Atherosclerosis
- Blood
  - ? Platelet aggregation

ANS
- ANS imbalance
  - ↑ SNS / ↓ PSNS

PM and/or constituents transmitted into blood
Pulmonary oxidative stress & inflammation
- Activation of lung ANS reflex arcs
- Sub-acute & Chronic

“Systemic spill-over”

Vascular Oxidative Stress and Inflammation
- Cellular inflammatory response (↑ activated WBCs, platelets, MPO)
- ↑ Cytokine expression/levels (↑ IL-1β, IL-6, TNF-α)
- ? ET, histamine, cell microparticles, oxidized lipids; ↓ anti-oxidants
- ↑ Adipokines (PAI-1, Resistin)
- Endothelial cell dysfunction/vasoconstriction, ↑ROS
- Atherosclerosis progression/plaque vulnerability
- ↑ Thrombogenicity (e.g. tissue factor)
- Insulin resistance, dyslipidemia, impaired HDL function
- ↑ Coagulation, thrombosis; ↓ fibrinolysis (e.g. PAI-1)
- Activated or Inflamed fat
- Direct actions
- Activated or Inflamed liver
- Acute phase response
- ↑ Clotting factors
- Fibrinogen, CRP
- Blood
  - Platelet aggregation
- Heart
  - ↓ HRV
  - ↑ Heart rate
  - ↑ Arrhythmia potential

Brook, R. D. et al. Circulation 2010;121:2331-2378
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