Differences in Inflammatory Responses to Exposures of Concentrated Ambient Particles in Susceptible Volunteers

Marc Riedl, M.D., M.S.
Assistant Professor of Medicine
Section of Clinical Immunology & Allergy
David Geffen School of Medicine
University of California, Los Angeles
Objectives

1) Provide background for adverse respiratory health effects of air pollution
2) Review current concepts of pollutant inflammatory effects and individual susceptibility factors
3) Present study design and results from recent human exposure study using concentrated air particles in susceptible individuals
4) Discuss implications of study findings for future research
Air Pollutants

“pestilential vapors and soot” described by Roman Empire

- Particulates
- Nitrogen Dioxide
- Sulfur Dioxide
- Carbon Monoxide
- Ozone
- Environmental Tobacco Smoke (ETS)
Particulate matter size distribution

- Respirable particles
- Thoracic particles
- PM$_{10-2.5}$ Coarse
- PM$_{2.5}$ Fine
- UFP (PM$_{0.1}$) Ultrafine
The Clinical Significance

- **The Epidemiology**
  - Observational studies convincingly link increased particulate air pollution levels with overall cardiorespiratory morbidity and mortality.
  - Numerous studies demonstrate association of increased particulate air pollution with:
    - asthma prevalence
    - asthma severity
    - asthma morbidity
    - asthma medication use
    - hospitalization for asthma
    - asthma mortality
    - allergic sensitization

Dose-response effect nearly linear; no threshold dose identified
Important questions on air pollution and respiratory diseases

Is there a link between air pollution and airway disease?

What are the mechanisms involved?

What confers susceptibility?

What can we do about it?
**Effects of DEP in Human Controlled Exposure Studies**

- **Healthy subjects**
  - ↑ Inflammatory cells in airways
  - ↑ Histamine levels in bronchial tissue
  - ↑ IL-6, IL-8
  - ↑ Expression of ICAM-1, VCAM-1
  - ↑ Airway resistance

- **Subjects with mild asthma**
  - ↑ Hyperresponsiveness to methacholine
  - ↑ Airway resistance
  - ↑ Sputum IL-6
  - No apparent increase in airway cellular inflammation
  - ↑ Epithelial IL-10 expression

- **Activation of redox-sensitive transcription factors:** NFκB, AP-1, JNK MAPK, p38 MAPK


*Stenfors N et al. Eur Respir J 2004*
Established Human Models Demonstrate DEP Pro-inflamatory and Pro-allergic Effects

1. Immediate phase response (minutes)
   - Increased allergen-induced histamine release and symptoms
2. Short-term response (hours)
   - Release of chemokines, cytokines and increased cellular inflammation
3. Intermediate-term response (days)
   - Enhanced total and allergen-specific IgE response to allergens
4. Long term response (days to weeks)
   - Enhanced primary allergic sensitization
The Adverse Health effects of Particulate Pollutants in the Airways is Related to the Biology of Oxidative Stress

Oxidative Stress Approach

1. PM contains pro-oxidative chemicals
2. PM chemicals generate ROS → Oxidative stress
3. Oxidative stress → cytoprotective response
4. Increased Oxidative stress → pro-inflammatory effects

And what to do about it?

Bowler and Crapo JACI 2002
Hierarchical oxidative stress model of cellular response to DEP exposure

Cell response pathway:
- Normal
- Anti-oxidant Defense
- Inflammation
- Toxicity

Signaling pathway:
- Nrf-2
- MAPK
- Mitochondrial perturbation

Genetic response:
- ARE
- AP-1
- PT pore

Regulatory factors:
- IL-4
- IL-5
- IL-13
- TNF-α
- ICAM-1/VCAM-1

Important questions on air pollution and respiratory diseases

Is there a link between air pollution and airway disease?

What are the mechanisms involved?

What confers susceptibility?

What can we do about it?
Identifying the Susceptible Individual

- Children, elderly, pre-existing respiratory conditions at risk
- Asthma severity may not be predictor for sensitivity to air pollution
- Inter-individual variability in inflammatory response to DEP


Gilliland et al; Lancet 363:119-25, 2004
Augmentation of allergen-IgE production by DEP is reproducible and intrinsic.


\[ r = 0.83, \ p < 0.01 \]
Challenge with DEP defines high and low responder populations

Susceptible phenotype
Enhanced susceptibility to DEP in GSTM1 null individuals

- Glutathione-S-transferases (GSTs)
  - Phase II metabolizing enzymes central to xenobiotic defense mechanisms
  - Protection against ROS generated oxidative stress
    - Detoxification of chemicals of particulate pollutants
    - Metabolism of reactive oxygen species

<table>
<thead>
<tr>
<th></th>
<th>GSTM null</th>
<th>GSTM (+)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgE (U/mL)</td>
<td>102.5</td>
<td>45.5</td>
<td>0.03</td>
</tr>
<tr>
<td>Histamine (nmol/L)</td>
<td>14.0</td>
<td>7.4</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Gilliland et al; Lancet 363:119-25, 2004
In Vitro Experimental Design

Human Bronchial Epithelial (HBE) cells

GSTM1 +

GSTM1 -

+DEP

+DEP

cytokine measurement at baseline and post-exposure to DEP
GSTM1 Function protective vs. DEP-induced inflammation

Wan et al. Unpublished data
GSTM1 Function protective vs. DEP-induced inflammation

Wan et al. Unpublished data
What are Concentrated Air Particles (CAPS)?

- “Real world” particles
- Chemically complex mixtures of soluble and insoluble components
- Components depend on geographic location and time
- Elements: carbon, sulfur, silicon, iron, calcium, zinc, nickel, copper, selenium, vanadium, etc.
- Reactive surface compounds: Sulfates, nitrates, acids, organics, polyaromatic hydrocarbons (PAH)
Human Exposure Studies of CAP in Asthma

Differences in Inflammatory Responses to Exposures of Concentrated Ambient Particles in Susceptible Volunteers

Air Resources Board Contract #05-341

Marc A. Riedl¹, William S. Linn²³, Kenneth W. Clark³, David Diaz-Sanchez⁴

¹University of California, Los Angeles – David Geffen School of Medicine
²University of Southern California Keck School of Medicine
³Los Amigos Research and Education Institute
⁴United States Environmental Protection Agency
Study Objective

To test hypothesis that individuals with certain ‘susceptibility factors’ will have heightened inflammatory and airway responses to exposure to concentrated ambient particles (CAPS)

- $GSTM_1$ null polymorphism
- underlying asthma
Study Design

- Single-blind randomized crossover study of controlled exposure to filtered air (FA) and to concentrated ambient fine particles (CAPS)
  - 2 hours, submaximal exercise for 15 min of every half-hour
  - Target CAPS concentration: 200 µg/m³
- Enrolled three distinct groups in exposure protocol:
  - 10 GSTM1-null asthmatics (mild-moderate)
  - 10 GSTM1-present asthmatics
  - 10 GSTM1-present healthy subjects.
- Comparison of resultant inflammatory and airway responses
- Cardiovascular measurements by 24-hour Holter monitor
Study Design

Schedule for exposures

Day 0    screening visit
Day 14   exposure to 200 ug/m³ CAPS*
Day 15   follow-up visit
Day 28   exposure to filtered air*
Day 29   follow-up visit

* Order of exposure to CAPS and FA randomized for each subject
Study Design

Pre-Exposure

- Symptom score sheet completed
- Initiation of Holter monitoring, ECG telemetry and pulse oximeter
- Vital signs
- 12-lead ECG at rest
- Venous blood drawing (20 cc)
- Nitric oxide measurement
- Nasal lavage
- Pre-exposure spirometry
- Urine collection

Post-Exposure

- Nasal Lavage
- Vital signs
- Spirometry
- Symptom score sheets
- Methacholine bronchoprovocation with spirometry
- Subject leaves laboratory with diary and Holter monitor

DAY 2

- Diary collected.
- Symptom score sheet
- Vital signs
- Venous blood drawing (20 cc)
- Urine collection
- Spirometry
- 12-lead ECG at rest
- Nitric oxide measurement
- Nasal Lavage
- Sputum induction
- Spirometry.
- Holter monitoring ends
Biologic Endpoints

- Vital Signs: Pulse, BP, O₂ saturation
- Bronchial reactivity
- Spirometry
- Exhaled NO, CO
- CV Holter: Indices of HRV, S-T voltage, repolarization
- Sputum: Differential cell counts, IgG, IgG₄, IgA, IgM, IgE; IL-4, IL-5, IL-8; GMCSF; IFN-γ, TNF-α
- Nasal Lavage: Differential cell counts, IgG, IgG₄, IgA, IgM, IgE; IL-4, IL-5, IL-8; IFN-γ, TNF-α
- Blood: C-reactive protein, Factor VII, von Willebrand factor, fibrinogen, IL-8, IgG, IgG₄, IgA, IgM, IgE
- Urine: 8-isoprostane
- Symptom score sheet
CAPS Exposure Methods

- Whole-body chamber: CAPS (PM2.5)
  - concentration of 200 µg/m³ monitored real time by nephelometer
  - controlled by diluting output of the ambient fine particle concentrator with varying amounts of filtered air
  - Concentrator resembles that used by EPA
  - Outdoor ambient air drawn from above the roof of the laboratory about 4 m above grade
  - Ambient particles are concentrated up to 9 times
CAPS Exposure Methods

- Important contributors to ambient PM at laboratory location include southern Los Angeles County background pollution, locally heavy surface-street traffic, diesel-truck-heavy I-710 freeway one mile west, port complex about 10 miles south
- Previous work has characterized neighborhood’s pollution and fine CAPS exposure atmospheres
- Nitrate, organic carbon, sulfate, and elemental carbon are major constituents of the fine CAPS
- Particle size distribution measurements performed with a micro-orifice uniform-deposit impactor (MOUDI) to determine contribution of ultrafine particles to CAPS

Filtered Air Exposures

- Filtered air (FA) exposure was used as control arm
- All FA exposures performed in the same chamber with same protocol except that ambient air was filtered by HEPA particle filtration
- Carbon monoxide, nitrogen oxides, sulfur dioxide, and ozone levels were monitored in incoming ambient air upstream of the particle concentrator during FA and CAPS exposures
- Prior testing has shown little difference between ambient and in-chamber measurements of gases
## Study Subjects Enrolled

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (Mean, range)</th>
<th>Gender (F/M)</th>
<th>Ethnicity (A/B/H/W)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asthma/GSTM (-)</td>
<td>30.4 (21-43)</td>
<td>9/1</td>
<td>1/0/6/3</td>
</tr>
<tr>
<td>Asthma/GSTM (+)</td>
<td>41.9 (20-55)</td>
<td>6/4</td>
<td>0/3/5/2</td>
</tr>
<tr>
<td>Healthy/GSTM (+)</td>
<td>32.0 (18-53)</td>
<td>7/3</td>
<td>0/1/8/1</td>
</tr>
</tbody>
</table>

*A Asian, B African-American, H Hispanic, W white non-Hispanic*
### Environmental Measurements (Mean ± SD) in Filtered Air Control Studies vs. Concentrated Fine Particle Exposures

<table>
<thead>
<tr>
<th>Measure</th>
<th>Filtered Air Controls</th>
<th>CAPS Exposures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass concentration, total filter (µg/m³)</td>
<td>35 ± 16</td>
<td>187 ± 42</td>
</tr>
<tr>
<td>Mass concentration, DataRAM (µg/m³)</td>
<td>13 ± 7</td>
<td>288 ± 55</td>
</tr>
<tr>
<td>Mass concentration, MOUDI (µg/m³)</td>
<td>16 ± 3 [b]</td>
<td>164 ± 39</td>
</tr>
<tr>
<td>O₃ (ppb)</td>
<td>23 ± 11</td>
<td>20 ± 11</td>
</tr>
<tr>
<td>NO₂ (ppb)</td>
<td>24 ± 14</td>
<td>34 ± 21</td>
</tr>
<tr>
<td>SO₂ (ppb)</td>
<td>1.8 ± 1.3</td>
<td>1.6 ± 0.8</td>
</tr>
<tr>
<td>CO (ppm)</td>
<td>1.6 ± 1.2</td>
<td>1.6 ± 1.1</td>
</tr>
<tr>
<td>Chamber temperature (°F)</td>
<td>71 ± 2</td>
<td>72 ± 2</td>
</tr>
<tr>
<td>Chamber relative humidity (%)</td>
<td>69 ± 11</td>
<td>70 ± 11</td>
</tr>
<tr>
<td>Outdoor temperature (°F)</td>
<td>76 ± 7</td>
<td>78 ± 7</td>
</tr>
<tr>
<td>Outdoor relative humidity (%)</td>
<td>44 ± 13</td>
<td>41 ± 7</td>
</tr>
</tbody>
</table>
Average Particle Mass vs. Size Range as Determined by MOUDI Sampling in CAPS Exposures
Average Particle Mass vs. Size Range as Determined by MOUDI Sampling in FA Exposures vs. CAPS Exposures
# Summary Statistics for Chemical Analyses of Particulate Samples from Exposures

<table>
<thead>
<tr>
<th>Species</th>
<th>Units</th>
<th>CAPs</th>
<th>FA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>(total mass)</td>
<td>µg/m³</td>
<td>186</td>
<td>43</td>
</tr>
<tr>
<td>EC</td>
<td>µg/m³</td>
<td>2.7</td>
<td>1.9</td>
</tr>
<tr>
<td>OC</td>
<td>µg/m³</td>
<td>32.4</td>
<td>8.0</td>
</tr>
<tr>
<td>Al</td>
<td>ng/l extract</td>
<td>30.1</td>
<td>16.7</td>
</tr>
<tr>
<td>K</td>
<td>ng/l extract</td>
<td>21.4</td>
<td>13.2</td>
</tr>
<tr>
<td>Ca</td>
<td>ng/l extract</td>
<td>34.7</td>
<td>20.2</td>
</tr>
<tr>
<td>Ti</td>
<td>ng/l extract</td>
<td>1.03</td>
<td>1.07</td>
</tr>
<tr>
<td>V</td>
<td>ng/l extract</td>
<td>0.46</td>
<td>0.31</td>
</tr>
<tr>
<td>Cr</td>
<td>ng/l extract</td>
<td>0.52</td>
<td>0.28</td>
</tr>
<tr>
<td>Fe</td>
<td>ng/l extract</td>
<td>36.3</td>
<td>19.1</td>
</tr>
<tr>
<td>Cu</td>
<td>ng/l extract</td>
<td>2.7</td>
<td>2.2</td>
</tr>
<tr>
<td>Zn</td>
<td>ng/l extract</td>
<td>8.0</td>
<td>3.5</td>
</tr>
<tr>
<td>Ba</td>
<td>ng/l extract</td>
<td>3.0</td>
<td>2.2</td>
</tr>
<tr>
<td>P</td>
<td>ng/l extract</td>
<td>3.6</td>
<td>7.0</td>
</tr>
<tr>
<td>S</td>
<td>ng/l extract</td>
<td>158</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>Healthy</td>
<td>Asthma GSTM1 +</td>
<td>Asthma GSTM1 null</td>
</tr>
<tr>
<td>----------------------</td>
<td>--------------</td>
<td>---------------</td>
<td>------------------</td>
</tr>
<tr>
<td>Symptom Score</td>
<td>1.1 (2.0)</td>
<td>2.7 (3.3)</td>
<td>1.6 (2.4)</td>
</tr>
<tr>
<td>FVC (ml)</td>
<td>4038 (850)</td>
<td>3786 (550)</td>
<td>4044 (796)</td>
</tr>
<tr>
<td>FEV$_1$ (ml)</td>
<td>3298 (686)</td>
<td>3002 (481)</td>
<td>3050 (622)</td>
</tr>
<tr>
<td>FEV$_1$/FVC (%)</td>
<td>81.9 (4.4)</td>
<td>79.5 (7.9)</td>
<td>75.6 (6.4)</td>
</tr>
<tr>
<td>BP systolic (mmHg)</td>
<td>115 (12)</td>
<td>109 (10)</td>
<td>111 (12)</td>
</tr>
<tr>
<td>BP diastolic (mmHg)</td>
<td>74 (11)</td>
<td>74 (9)</td>
<td>73 (12)</td>
</tr>
<tr>
<td>SaO$_2$ (%)</td>
<td>98.5 (0.9)</td>
<td>98.2 (2.0)</td>
<td>98.4 (1.3)</td>
</tr>
<tr>
<td>FeNO (ppb)</td>
<td>26 (13)</td>
<td>50 (53)</td>
<td>42 (30)</td>
</tr>
<tr>
<td>FeCO (ppm)</td>
<td>1.3 (0.9)</td>
<td>1.3 (1.2)</td>
<td>1.0 (0.8)</td>
</tr>
</tbody>
</table>
Summary of Mixed-Model Analyses of Physiology and Symptom Data

<table>
<thead>
<tr>
<th>Measure of Response</th>
<th>Significant (P &lt; 0.05) Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptom score during exp.</td>
<td>increase from pre-exposure, larger in GSTM1-null</td>
</tr>
<tr>
<td>Symptom score after exp.</td>
<td>increase from pre-exposure in GSTM1-null only</td>
</tr>
<tr>
<td>ΔFVC post - pre or day 2 - pre</td>
<td>(none)</td>
</tr>
<tr>
<td>ΔFEV\textsubscript{1} post – pre or day 2 - pre</td>
<td>(none)</td>
</tr>
<tr>
<td>ΔBP systolic post – pre (mmHg)</td>
<td>decrease from pre-exposure, less in GSTM1-null</td>
</tr>
<tr>
<td>ΔBP systolic day 2 – pre (mmHg)</td>
<td>(none)</td>
</tr>
<tr>
<td>ΔBP diastolic post–pre or d2-pre</td>
<td>(none)</td>
</tr>
<tr>
<td>ΔSaO\textsubscript{2} post – pre (%)</td>
<td>(none)</td>
</tr>
<tr>
<td>ΔFeNO post – pre (ppb)</td>
<td>increase after CAPS relative to FA</td>
</tr>
<tr>
<td>ΔFeNO day 2 – pre (ppb)</td>
<td>(none)</td>
</tr>
<tr>
<td>ΔFeCO post–pre or day 2 – pre</td>
<td>(none)</td>
</tr>
</tbody>
</table>
Mean Change in SBP pre- to post-exposure, FA vs. CAPS, for Each Group and for All Subjects Pooled
Mean change in log-transformed FeNO pre- to post-exposure, FA vs. CAPS, for Each Group and for All Subjects Pooled
## Correlations of Response Measures with Exposure Measures

<table>
<thead>
<tr>
<th></th>
<th>FVC</th>
<th>FEV₁</th>
<th>BP systolic</th>
<th>BP diastolic</th>
<th>SaO₂</th>
<th>Symptom Score</th>
<th>FeNO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration (filter sample)</td>
<td>-0.01</td>
<td>+0.04</td>
<td>-0.28</td>
<td>+0.19</td>
<td>+0.25</td>
<td>-0.20</td>
<td>-0.15</td>
</tr>
<tr>
<td>Concentration (DataRAM)</td>
<td>+0.07</td>
<td>+0.19</td>
<td>-0.11</td>
<td>-0.06</td>
<td>-0.00</td>
<td>-0.29</td>
<td>+0.11</td>
</tr>
<tr>
<td>NO₂</td>
<td>+0.15</td>
<td>+0.34</td>
<td>-0.04</td>
<td>-0.29</td>
<td>+0.33</td>
<td><strong>-0.63</strong> (P &lt; .001)</td>
<td>-0.12</td>
</tr>
<tr>
<td>Chamber Temperature</td>
<td>-0.02</td>
<td>-0.17</td>
<td>-0.02</td>
<td>+0.14</td>
<td>+0.05</td>
<td>+0.13</td>
<td>+0.14</td>
</tr>
</tbody>
</table>
Mean Percentage of Monocytes, Lymphocytes, and Neutrophils in Induced Sputum, by Susceptibility Group and Induction Condition
Mean Concentration of IL-4 in Induced Sputum, by Susceptibility Group and Induction Condition
### Percentage of Subjects with Detectable Concentrations of Usually Nondetectable Biomarkers in Sputum: Comparison between Filtered Air and CAPS

<table>
<thead>
<tr>
<th></th>
<th>% after FA</th>
<th>% after CAPS</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eosinophils</td>
<td>27</td>
<td>17</td>
<td>0.16</td>
</tr>
<tr>
<td>Immunoglobulin E</td>
<td>7</td>
<td>3</td>
<td>0.38</td>
</tr>
<tr>
<td>Interferon-gamma</td>
<td>7</td>
<td>17</td>
<td>0.16</td>
</tr>
<tr>
<td>GMCSF</td>
<td>10</td>
<td>17</td>
<td>0.23</td>
</tr>
<tr>
<td>TNF-alpha</td>
<td>13</td>
<td>27</td>
<td>0.11</td>
</tr>
</tbody>
</table>
Estimated Mean IgG4 in Nasal Lavage Fluid, as a Function of Time, by Group and Exposure Atmosphere

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>CAPs</th>
<th>Time</th>
<th>Interaction</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG4</td>
<td>.002</td>
<td></td>
<td></td>
<td>C*T .009</td>
<td>healthy &gt; asthma(0) &gt; asthma(+) down after FA, up after CAPS</td>
</tr>
</tbody>
</table>

**Diagram:***
- **Axes:** log (IgG4) [orig. units ng/ml] on the y-axis and time (Pre, 2h Post, 22h Post) on the x-axis.
- **Groups:**
  - **Asth 0 FA**
  - **Asth 0 CAPS**
  - **Asth + FA**
  - **Asth + CAPS**
  - **Heal + FA**
  - **Heal + CAPS**
  - **ALL FA**
  - **ALL CAPS**

**Legend:**
- Healthy > asthma(0) > asthma(+) down after FA, up after CAPS

**Estimated SE:** 0.14 for all, 0.21 for separate groups
**Pairwise Rank Correlations between Exposure and Sputum Response Variables**

<table>
<thead>
<tr>
<th></th>
<th>Mass</th>
<th>Fe</th>
<th>Cu</th>
<th>Cr</th>
<th>Zr</th>
<th>Ba</th>
<th>% Lym</th>
<th>IL5</th>
<th>IgA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe</td>
<td>+.32</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>+.24</td>
<td>+.47</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cr</td>
<td>+.64</td>
<td>+.60</td>
<td>+.31</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zr</td>
<td>+.36</td>
<td>+.44</td>
<td>+.15</td>
<td>+.35</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ba</td>
<td>+.32</td>
<td>+.87</td>
<td>+.39</td>
<td>+.62</td>
<td>+.54</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Lym</td>
<td>-.20</td>
<td>-.36</td>
<td>-.43</td>
<td>-.28</td>
<td>-.11</td>
<td>-.32</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL5</td>
<td>+.17</td>
<td>-.37</td>
<td>-.02</td>
<td>-.12</td>
<td>-.30</td>
<td>-.40</td>
<td>+.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgA</td>
<td>+.41</td>
<td>-.14</td>
<td>+.01</td>
<td>+.37</td>
<td>-.13</td>
<td>-.14</td>
<td>+.07</td>
<td>+.19</td>
<td></td>
</tr>
<tr>
<td>IgG4</td>
<td>+.43</td>
<td>-.10</td>
<td>-.05</td>
<td>+.40</td>
<td>-.06</td>
<td>+.02</td>
<td>+.04</td>
<td>+.02</td>
<td>+.68</td>
</tr>
</tbody>
</table>

*P < 0.05, **P < 0.01, ***P < 0.001
Heart Rate Variability

<table>
<thead>
<tr>
<th>Variable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean RR interval</td>
</tr>
<tr>
<td>Calculated HR</td>
</tr>
<tr>
<td>log (pNN50)</td>
</tr>
<tr>
<td>log (normalized high-frequency power)</td>
</tr>
<tr>
<td>Median ST voltage V5</td>
</tr>
<tr>
<td>Mean lead II QTcB</td>
</tr>
<tr>
<td>T amplitude</td>
</tr>
<tr>
<td>log (SD QTcB)</td>
</tr>
<tr>
<td>log (TD/II norm variance)</td>
</tr>
<tr>
<td>TD/II variability index</td>
</tr>
</tbody>
</table>

![T-wave Complexity Chart](chart_image)
Overall Conclusions

• No demonstrated clear robust differences between exposure responses of normal vs. susceptible subjects
• With exception of increase in FeNO for all groups, CAPS exposure as performed in this study does not appear to produce a robust inflammatory respiratory or systemic response in human subjects
• Study did not find that subjects with asthma and/or GSTM1 null genotype were more susceptible to the inflammatory effects of CAPS exposure
Summary of Findings for Biologic Endpoints

- Significant baseline differences in FeNO for asthmatic vs. healthy subjects
- Trend but nonsignificant difference in FEV1/FVC for asthmatics vs. healthy subjects: mild asthma in study population
- Few differences in responses attributable to exposure conditions (FA vs. CAPS) or between susceptibility groups
Changes in FeNO

- Increases in CAPS exposure vs. FA
- Did not vary significantly based on GSTM or asthma status, though mean increase greater in asthma vs. healthy groups
- Previously reported in observational studies
- No previous controlled fine CAPS study for comparison
- Coarse CAPS, UF CAPS, DEP exposures have not shown consistent increases in FeNO
Sputum Biomarkers

- GSTM1-positive asthmatics had significantly higher levels of sputum PMN counts, sputum IgA, and lower levels of sputum monocytes compared to other groups.
- Explanation for finding in GSTM1-positive vs. GSTM1-null asthmatics not clear.
- Not significantly affected by exposure conditions.
Sputum IgA

- Significant group differences for sputum IgA with CAPS vs. FA
- Healthy subjects showed a significant increase while GSTM1-positive asthmatics showed little change and GSTM-null asthmatics showed a mild decrease with CAPS exposure
- Overall group variation with CAPS exposure approached statistical significance (p=0.06)
Sputum IgA

- Recently recognized anti-inflammatory role of mucosal and systemic IgA
- Speculative link between IgA and the anti-oxidant role of GSTM1 that could potentially explain association
- Requires additional study to investigate the association and/or mechanism

Sputum IL-4

- Asthmatic groups had higher mean sputum IL-4 levels compared to the healthy controls
- All groups showed decreases in mean IL-4 levels after either exposure (CAPS or FA) relative to baseline
- Contrasts with previous data from DEP studies suggesting that particulate air pollutants induce increased IL-4 production from T-cells
Blood and Urine Biomarkers

- Blood and urine biomarkers did not show exposure differences attributable to CAPS.
- Previous human studies of CAPS exposure effects on various serologic biomarkers have yielded variable results from no significant change to mild increases in fibrinogen, D-dimer, and IL-8 (latter two effects observed with concentrated ultrafine particles).

Heart Rate Variability

- HRV recognized as an important cardiovascular outcome
- Reduced HRV considered a prognostic marker for the development of cardiac arrhythmia
- HRV changes in our study that appeared attributable to CAPS exposure across all groups included a mild decrease in HR and decreased T-wave complexity and variability
Heart Rate Variability

- Our results inconsistent with previous reports of increases in both HR and T-wave complexity/variability after particle exposure
- Differences with regard to particle size (ultrafine) and study population (ischemic heart disease)

Exposure Analysis

- Exposures Adequate?
- Employed well-established protocols and equipment which have been used successfully for a number of previous exposure studies
- Air monitoring results showed experimental exposures to fine CAPS close to target concentration of 200 µg/m³
Particle Characteristics

- EC/OC results consistent with previous CAPS exposures
- PAH levels detected in particles appear relatively low with many filter samples below limit of detection for a number PAHs
- Reduced PAH content may be a contributing factor to findings if particle redox activity strongly correlated to PAH
- Detectable but lower than expected levels of a number of transition metals and elements believed to be important in the generation of ROS and inflammation
- No reason to believe collected air particles in region have changed substantially compared to previous studies at our site
- Qualitatively, chemical composition of CAPS used in our study may differ from those in other CAPS exposure studies
Study Limitations

- Individuals in asthma groups were clinically mild-moderate
  - Subjects not taking inhaled or systemic corticosteroids and required to have baseline FEV1 >70%
- Strength of conclusion limited by study power
  - Designed to detect a 3% exposure-related reduction in FEV1 (smallest clinically meaningful FEV1 change) with power of 0.8 using a one-tail test with alpha = 0.05 and N = 10
  - More subtle changes in biomarkers could have gone undetected
Study Limitations

- Many response variables measured in relatively few subjects with biologic variability due to personal environmental stresses outside the confines of the experiment
- Possible that spurious statistically significant differences will be found
  - Due to uncontrolled and unmeasured intercurrent interferences
  - Due to a few "significant" differences found by chance in any large collection of statistical test results
Study Limitations

- Biologic heterogeneity between human individuals with considerable inter- and intra-subject variability over time
- Variations due to age, diet, genetic background, activity level, ambient exposures, and disease history
  - Obesity as emerging factor with potential impact on individual response to particulate matter, not included in original hypotheses
  - Susceptibility groups had similar numbers of overweight or obese subjects (7 asthmatic GSTM1 null, 7 asthmatic GSTM1 present, 8 healthy GSTM1 present)

Baja ES, et al. Environ Health Perspect. 2010
Study Limitations

- Important co-factors, genetic or otherwise, may modulate the response to particle exposure or oxidative stress in the absence of GSTM1
  - other Phase II antioxidant enzymes
  - cytoprotective mechanisms may play a role in reducing cellular oxidative stress
Considerations for Future Studies

- Larger-scale experiments with increased power
- Alternatives to spirometric changes as primary endpoints
- Increased CAPS exposure (higher concentration and/or greater duration)
- Ethical inclusion of more clinically severe asthmatics
- Consideration of additional genetic and host co-factors (i.e. dietary) that may modulate inflammatory response to oxidative stress
- Inclusion of FeNO measurement in future fine CAPS exposures to determine changes, significance
ACKNOWLEDGEMENTS

David Diaz-Sanchez, PhD

Los Amigos Research and Education Institute
  William Linn
  Kenneth Clark
  LAREI Research Staff

UCLA
  Hema Shah

USC
  Kiros Berhane, PhD