Baseline Repeated Measures from Controlled Human Exposure Studies: Associations between Ambient Air Pollution Exposure and the Systemic Inflammatory Biomarkers IL-6 and Fibrinogen

Aaron M.S. Thompson,1,2 Antonella Zanobetti,3 Frances Silverman,1,2 Joel Schwartz,3 Brent Coull,3 Bruce Urch,1 Mary Speck,1,2 Jeffrey R. Brook,4 Michael Manno,1,2 and Diane R. Gold3

1Gage Occupational and Environmental Health Unit, University of Toronto, Toronto, Ontario, Canada; 2St. Michael’s Hospital, Toronto, Ontario, Canada; 3Department of Environmental Health, Harvard School of Public Health, Boston, Massachusetts, USA; 4Environment Canada, Toronto, Ontario, Canada

INTRODUCTION: Systemic inflammation may be one of the mechanisms mediating the association between ambient air pollution and cardiovascular morbidity and mortality. Interleukin-6 (IL-6) and fibrinogen are biomarkers of systemic inflammation that are independent risk factors for cardiovascular disease.

OBJECTIVE: We investigated the association between ambient air pollution and systemic inflammation using baseline measurements of IL-6 and fibrinogen from controlled human exposure studies.

METHODS: In this retrospective analysis we used repeated-measures data in 45 nonsmoking subjects. Hourly and daily moving averages were calculated for ozone, nitrogen dioxide, sulfur dioxide, and particulate matter ≤ 2.5 µm in aerodynamic diameter (PM2.5). Linear mixed-model regression determined the effects of the pollutants on systemic IL-6 and fibrinogen. Effect modification by season was considered.

RESULTS: We observed a positive association between IL-6 and O3 (0.31 SD per O3 interquartile range (IQR); 95% confidence interval (CI), 0.08–0.54) and between IL-6 and SO2 (0.25 SD per SO2 IQR; 95% CI, 0.06–0.43). We observed the strongest effects using 4-day moving averages. Responses to pollutants varied by season and tended to be higher in the summer, particularly for O3 and PM2.5. Fibrinogen was not associated with pollution.

CONCLUSIONS: This study demonstrates a significant association between ambient pollutant levels and baseline levels of systemic IL-6. These findings have potential implications for controlled human exposure studies. Future research should consider whether ambient pollution exposure before chamber exposure modifies IL-6 response.

Effect estimates for fibrinogen were presented as the linear increase in fibrinogen (grams per liter) per each pollutant's IQR.

Data were analyzed using the statistical package SAS version 9.1 (SAS Institute Inc., Cary, NC, USA).

Results

Study population. The study population consisted of 45 participants (22 males, 23 females) with a mean of 3.9 (range, 1–6) repeated blood samples, mean age of 26.6 years (range, 19–48 years), and mean BMI of 22.7 (range, 17.8–29.9). Ten of the 45 subjects had a history of well-controlled asthma.

Inflammatory markers. Table 1 displays summary data on the inflammatory markers by study and participant characteristics. A total of 163 samples of IL-6 and 160 samples of fibrinogen were available for analysis. IL-6 and fibrinogen were moderately correlated (Spearman correlation coefficient = 0.21, p = 0.01).

Table 1. Inflammatory markers by study and participant characteristics.

<table>
<thead>
<tr>
<th>Study/characteristic</th>
<th>No. of subjects</th>
<th>Mean fibrinogen (range) (g/L)</th>
<th>Mean IL-6 (range) (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Study A</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All participants</td>
<td>23</td>
<td>2.30 (0.97–3.80)</td>
<td>0.64 (0.21–2.67)</td>
</tr>
<tr>
<td>Female</td>
<td>12</td>
<td>2.47 (1.50–3.80)</td>
<td>0.50 (0.02–2.40)</td>
</tr>
<tr>
<td>Male</td>
<td>11</td>
<td>2.09 (0.97–2.93)</td>
<td>0.82 (0.00–2.67)</td>
</tr>
<tr>
<td>Nonasthmatic</td>
<td>13</td>
<td>2.29 (0.97–3.53)</td>
<td>0.75 (0.00–2.67)</td>
</tr>
<tr>
<td>Asthmatic</td>
<td>10</td>
<td>2.32 (1.38–3.80)</td>
<td>0.50 (0.05–1.84)</td>
</tr>
<tr>
<td><strong>Study B</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All participants</td>
<td>22</td>
<td>2.54 (1.39–4.55)</td>
<td>3.06 (3.97–146.20)</td>
</tr>
<tr>
<td>Female</td>
<td>11</td>
<td>2.83 (1.87–3.46)</td>
<td>48.93 (4.52–138.30)</td>
</tr>
<tr>
<td>Male</td>
<td>11</td>
<td>2.35 (1.39–4.55)</td>
<td>33.38 (3.97–146.20)</td>
</tr>
</tbody>
</table>

Study A analyzed IL-6 samples using ELISA plates, whereas study B used the Luminex bead assay system. IL-6 data were transformed to standard scores (Z-scores) before compilation into single data set.
observed significant correlations among NO₂, SO₂, CO, and PM₁.₅. NO₃, SO₂, and CO were negatively correlated with O₃.

**Regression results.** IL-6 was positively correlated with each of the pollutants investigated and reached statistical significance at the 95% level with O₃ and SO₂ (Figure 1). Associations increased with longer moving averages, with statistical significance being reached with the 1- to 6-day moving average for O₃ and the 4- and 5-day moving average for SO₂. We observed similar trends for NO₂ and PM₁.₅. We observed the strongest association between IL-6 and O₃ using the 4-day moving average: a 0.31 SD increase in IL-6 per O₃ IQR [95% confidence interval (CI), 0.08–0.54]. The 4-day moving average for SO₂ showed a 0.25 SD increase in IL-6 per SO₂ IQR (95% CI, 0.06–0.43). The positive association between IL-6 and each pollutant declined with moving averages longer than 6 days. Fibrinogen was not significantly correlated with any of the pollutants investigated, and we found no trend for increasing moving averages (Figure 2).

**Effect modification by season.** Pollution associations with level of IL-6 varied by season, with higher effects of O₃ on IL-6 in the spring and summer and higher effects of PM₁.₅ in the summer. NO₂ associations with IL-6 were elevated both in summer (marginally) and in winter. The significance of the effect modification increased with moving averages up to 3 days, with the strongest effects occurring using 2-day moving averages (Figure 3). We observed no effect modification by season for fibrinogen and the investigated pollutants.

**Table 2.** Pollutant and meteorologic data by season, 14 July 1999 to 27 March 2006 (mean ± SD).

<table>
<thead>
<tr>
<th>Pollutant</th>
<th>Spring</th>
<th>Summer</th>
<th>Autumn</th>
<th>Winter</th>
<th>Annual</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO₂ (ppb)</td>
<td>24.98 ± 13.36</td>
<td>20.83 ± 11.54</td>
<td>22.61 ± 11.19</td>
<td>26.78 ± 10.70</td>
<td>23.79 ± 11.95</td>
</tr>
<tr>
<td>SO₂ (µg/m³)</td>
<td>3.09 ± 11.40</td>
<td>2.95 ± 13.62</td>
<td>3.61 ± 3.60</td>
<td>4.60 ± 4.67</td>
<td>3.57 ± 3.61</td>
</tr>
<tr>
<td>PM₁.₅ (µg/m³)</td>
<td>7.52 ± 7.29</td>
<td>12.34 ± 11.26</td>
<td>7.70 ± 8.05</td>
<td>6.12 ± 4.98</td>
<td>8.46 ± 8.57</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>3.74 ± 2.76</td>
<td>21.11 ± 4.88</td>
<td>10.93 ± 7.22</td>
<td>-2.85 ± 6.17</td>
<td>9.22 ± 10.82</td>
</tr>
<tr>
<td>Humidity (%)</td>
<td>64.20 ± 17.83</td>
<td>66.69 ± 16.39</td>
<td>73.31 ± 15.13</td>
<td>74.25 ± 12.41</td>
<td>69.58 ± 16.16</td>
</tr>
</tbody>
</table>

**Table 3.** Meteorologic and pollutant data (daily averages): Spearman rank correlation coefficients for the study period 14 July 1999 to 27 March 2006.

<table>
<thead>
<tr>
<th>CO</th>
<th>NO₂</th>
<th>O₃</th>
<th>SO₂</th>
<th>PM₁.₅</th>
<th>Humidity</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO</td>
<td>1.00</td>
<td>0.49</td>
<td>-0.24</td>
<td>0.43</td>
<td>0.25</td>
<td>-0.06</td>
</tr>
<tr>
<td>NO₂</td>
<td>1.00</td>
<td>-0.53</td>
<td>0.44</td>
<td>0.41</td>
<td>-0.11</td>
<td>-0.19</td>
</tr>
<tr>
<td>O₃</td>
<td>1.00</td>
<td>-0.19</td>
<td>0.03**</td>
<td>-0.23</td>
<td>-0.32</td>
<td>-0.09</td>
</tr>
<tr>
<td>SO₂</td>
<td>1.00</td>
<td>0.45</td>
<td>-0.10</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>PM₁.₅</td>
<td>1.00</td>
<td>0.45</td>
<td>-0.10</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Humidity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All correlations p < 0.01 unless otherwise specified. **p < 0.148.

**Discussion**

In measurements taken before controlled chamber exposure to pollution, study participants had elevated systemic levels of IL-6 in response to elevations in the previous 4-day cumulative averages of ambient O₃ and SO₂ levels. IL-6 responses tended to be higher in the spring and summer for O₃ and in the summer for PM₁.₅. In Toronto these seasons when open windows may allow more penetration of O₃ (Ren et al. 2006; Stafoggia et al. 2008) and when mixtures of O₃ and particulate pollution may be more prominent.

The observed correlations for the pollutants and meteorologic parameters in this study (Table 3) were consistent with the primary sources of the pollutants in the study region and established atmospheric chemical processes. The negative correlation between O₃ and NO₂ is in keeping with the process of nitric oxide scavenging O₃ in the atmosphere and the photodissociation of NO₂ to nitric oxide and O₃ (Baneey and Goisaugh 2002). The correlation among CO, SO₂, and NO₂ suggests traffic as the common source. This premise is also supported by the fact that the hour-of-day effect for each pollutant peaked in the mornings and, to a lesser extent, the afternoons and the day-of-week effect showed a drop on weekends.

The literature documents pollutant-associated elevations in pulmonary IL-6 in healthy subjects (in vitro) and tissue (in vitro) (Aarsalane et al. 1995; Carter et al. 1997; Devlin et al. 1991; Nordenhäll et al. 2000; Quay et al. 1998; Rückerl et al. 2007). Elevations in systemic IL-6 with elevated PM pollution have also been found in subjects with preexisting chronic inflammatory conditions (Dubowsky et al. 2006). Our study adds to the conclusion that pollution may increase systemic inflammation even in young healthy subjects. In young people, beyond the acute subclinical effects, recurrent low-grade acute inflammatory responses to pollution may ultimately have implications for the evolution of atherosclerosis and other processes influenced by inflammation (Künzli et al. 2005).

Fibrinogen was not significantly associated with any of the pollutants considered in this investigation. It may be that ambient levels of PM₁.₅ in this study were too low to induce a significant effect. This hypothesis is supported by the literature, which has reported no significant association between ambient pollution and fibrinogen at low levels of exposure (Pope et al. 2004; Rückerl et al. 2006) but significant associations with high exposures such as during high air pollution episodes or controlled human exposure studies (Ghiu et al. 2000).

In our study of young healthy subjects, we found that cumulative exposure to pollution over longer periods of time (3–6 days) was
associated with the strongest associations with elevated IL-6. In elderly subjects with diabetes or obesity, longer cumulative averages also resulted in the greatest effects on inflammation in a study of PM2.5 effects on CRP, IL-6, and white blood cells (Dubowsky et al. 2006). Rückerl et al. (2007) found an increase in IL-6 associated with particle number concentration, with a shorter lag of 12–17 hr. It may be that because all of our blood draws took place at 1000 hours, the 2- to 12-hr moving averages represent times when most subjects were in their homes; this may have attenuated the more immediate effects of outdoor pollution levels. Alternatively, at these levels of pollution, a longer cumulative exposure may be needed for this inflammatory response.

A limitation of this study was the small sample size, which limited power to test for interactions. Additional limitations include the absence of indoor home monitoring and the use of fixed-site ambient pollution monitoring, which may result in exposure misclassification, particularly for ambient pollutants that have strong local sources (Briggs et al. 1997). Each of these limitations would be expected to bias the results toward the null. Although we found that the association of many of the pollutants with elevated IL-6 was greater in the summer, correlation among the pollutants and limited numbers of observations made it difficult to evaluate which of the pollutants, or which mixture of pollutants, was leading to the increased inflammatory response. We could not confirm the associations of ambient pollution with inflammation by using additional complementary endpoints (e.g., CRP, tumor necrosis factor-α). We are able to describe relative but not absolute changes of IL-6 levels in response to pollution because we transformed the data to Z-scores in order to combine data from two separate studies.

The potential implications of the findings of this study extend beyond demonstrating that ambient pollutant exposure has a significant effect on baseline systemic levels of IL-6. Knowledge regarding the effect of prior ambient pollution exposure in baseline evaluation of systemic inflammation in human chamber exposure studies is important for interpretation of the results of such studies. Ning et al. (2004), using in vitro studies, demonstrated that “priming” of lung epithelial cells with inflammatory mediators before exposure to fine CAPs resulted in a greater inflammatory response. By analogy, priming of baseline inflammatory status by prior ambient air pollution exposure could modify the response of human subjects to controlled pollutant exposure in chamber studies. Such effect modification could lead to nondifferential misclassification bias (trials are conducted in random order), resulting in an underestimation of the true effect that pollutant exposure has on the induction of inflammation in humans.

Alternatively, ambient pollution could potentially prime and amplify the response to exposure in the chamber. A third possibility is that adaptation through ambient exposures to pollution could dampen certain immunologic or physiologic responses to acute chamber exposures (Bell et al. 1977). Finally, if prior exposure to ambient pollution were to equally affect pre- and postexposure measurements, then there would be much less concern about taking prior exposures to ambient pollutants into effect when doing controlled exposure studies. Investigation of the effects of cumulative ambient exposures to pollutants on responses to controlled human chamber exposure to pollutants will help in the interpretation of these studies.

**Conclusion**

Our results support previous findings of an association between ambient pollution and IL-6. In our analysis, exposure to ambient levels of O3 and SO2 was positively and significantly associated with a systemic inflammatory response as measured by systemic levels of IL-6. The association between IL-6 and O3 and SO2 demonstrated a cumulative lag effect with the strongest effects observed using 3- to 5-day moving averages. Pollution effects varied by season. Fibrinogen levels were not correlated with any of the investigated pollutants. Having demonstrated an effect of ambient pollutant exposure on baseline systemic levels of IL-6, future research should focus on whether ambient pollution exposure modifies the effect of inflammatory responses to controlled pollution exposures human chamber studies.

**Figure 2.** Associations between fibrinogen (g/L) and O3 (A), SO2 (B), and NO2 (C) per 1 ppb increase, and PM2.5 (D) per 1 µg/m3 increase. All models were adjusted for age, sex, BMI, asthma, day of the week, season, temperature (24-hr moving average), and relative humidity (24-hr moving average). Data are mean changes in fibrinogen with 95% CIs.

**Figure 3.** Effect modification of associations between IL-6 and CO, NO2, O3, SO2, and PM2.5 by season using 2-day moving averages. All models were adjusted for age, sex, BMI, asthma, day of the week, temperature (24-hr moving average), and relative humidity (24-hr moving average). Data are mean changes in IL-6 SD with 95% CIs for an IQR increase in each pollutant.
References


