Controlled Exposure of Healthy Young Volunteers to Ozone Causes Cardiovascular Effects

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Background—Recent epidemiology studies have reported associations between short-term ozone exposure and mortality. Such studies have previously reported associations between airborne particulate matter pollution and mortality, and support for a causal relationship has come from controlled-exposure studies that describe pathophysiological mechanisms by which particulate matter could induce acute mortality. In contrast, for ozone, almost no controlled-human-exposure studies have tested whether ozone exposure can modulate the cardiovascular system.

Methods and Results—Twenty-three young healthy individuals were exposed in a randomized crossover fashion to clean air and to 0.3-ppm ozone for 2 hours while intermittently exercising. Blood was obtained immediately before exposure, immediately afterward, and the next morning. Continuous Holter monitoring began immediately before exposure and continued for 24 hours. Lung function was performed immediately before and immediately after exposure, and bronchoalveolar lavage was performed 24 hours after exposure. Immediately after ozone exposure, we observed a 98.9% increase in interleukin-8, a 21.4% decrease in plasminogen activator inhibitor-1, a 51.3% decrease in the high-frequency component of heart rate variability, and a 1.2% increase in QT duration. Changes in interleukin-1B and plasminogen activator inhibitor-1 were apparent 24 hours after exposure. In agreement with previous studies, we also observed ozone-induced drops in lung function and an increase in pulmonary inflammation.

Conclusions—This controlled-human-exposure study shows that ozone can cause an increase in vascular markers of inflammation and changes in markers of fibrinolysis and markers that affect autonomic control of heart rate and repolarization. We believe that these findings provide biological plausibility for the epidemiology studies that associate ozone exposure with mortality.

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Key Words: autonomic nervous system ■ fibrinolysis ■ inflammation

Ozone, a ubiquitous air pollutant, has been studied more extensively than perhaps any other environmental toxicant. Numerous articles have described the health effects associated with exposure to ozone. However, until recently, nearly all of them have focused on characterizing respiratory effects. Although epidemiology studies have associated exposure to particulate matter (PM) with acute mortality and morbidity, only recently have they found associations between ozone and mortality. It has been challenging to disentangle the effects of ozone from those of PM in these studies because the 2 pollutants are often closely correlated temporally and geographically. Despite this, there is an emerging body of studies that report robust associations between ozone and cardiovascular mortality

Clinical Perspective on p 111

Unlike for PM, relatively little work has established biological plausibility for the observed association between ozone exposure and mortality. Epidemiological studies have shown that PM levels are associated with increased hospitalizations and alterations in markers of cardiovascular risk such as arrhythmia, changes in heart rate variability (HRV), vascular inflammation, and endothelial cell dysfunction. Evidence for a causal link between PM and mortality has been bolstered by controlled-exposure studies to PM that have described pathophysiological processes by which short-term PM exposure could cause death and the molecular pathways that underlie these processes. However, this type of coherence between epidemiological, clinical, and molecular studies does not yet exist for ozone.
Indeed, only a handful of controlled-exposure studies have examined cardiovascular effects resulting from ozone exposure. Foster et al.\(^1\) found a reduction in serum levels of the free radical scavenger α-tocopherol after ozone exposure, and Gong et al.\(^2\) observed an increase in alveolar-to-arterial PO\(_2\) gradient that could be due to impaired alveolar-arterial oxygen transfer. Some animal toxicology studies have reported increased systolic blood pressure and heart rate,\(^3\) which are thought to be related to a drop in core temperature in rats exposed to ozone.\(^4\) Chuang et al.\(^5\) also reported an ozone-induced increase in vascular oxidative stress and mitochondrial DNA damage, and Perepu et al.\(^6\) observed enhanced sensitivity to myocardial ischemia reperfusion injury in rats exposed to ozone.\(^7\)

Taken as a whole, there is weak coherence between the recent epidemiological associations of cardiovascular mortality and morbidity and ozone exposure, and there are few controlled exposure studies that have attempted to provide biological plausibility and possible biological mechanisms that explain the association between ozone and acute mortality. We hypothesized that if such an association is real, then the effects of ozone exposure should be apparent outside the airways and should be manifested in systemic changes in the cardiac or vascular systems. To test this, we characterized cardiovascular changes after exposing healthy young volunteers to ozone. Here, we report changes in vascular markers of fibrinolysis, markers of inflammation, HRV, and repolarization.

**Methods**

**Study Population**

Participants were recruited under a contract to the Westat Corp, which identified potential subjects through its Web site, newspaper advertising, and targeted calls to qualified subjects who had completed other Environmental Protection Agency studies. A total of 23 volunteers completed the study. The median age was 28.8 years (youngest participant was 19 years of age, oldest was 33 years of age); there were 10 male and 3 female participants (21 whites, 1 Hispanic, 1 black). The use of young healthy subjects could be seen as a limitation of the study because it is not the population that might be expected to respond in a clinically significant manner to ozone. However, for safety reasons, we chose not to study a more at-risk population. The ozone-induced effects reported here could conceivably be greater in at-risk individuals or, even if the same, could potentially place them at greater risk than healthy young individuals.

Mean resting heart rate of the study population was 68.3 bpm (51–88 bpm). Mean diastolic blood pressure was 74.9 mm Hg (64–86 mm Hg) and mean systolic blood pressure was 123.3 mm Hg (103–136 mm Hg). Participants were free of cardiopulmonary diseases and allergies as determined by a detailed medical history and physical examination. All subjects had forced expiratory volume in the first second of expiration (FEV\(_1\)) and forced vital capacity (FVC) baseline values of at least 80% predicted for height and age and were lifetime nonsmokers, except for 1 subject who had smoked half of a pack of cigarettes per year until 1998. Subjects were informed of the procedures and potential risks and signed an informed consent. The protocol and consent forms were approved by the University of North Carolina School of Medicine Committee on the Protection of the Rights of Human Subjects and the US Environmental Protection Agency.

**Study Design**

This was a randomized single-blind crossover study in which each subject was exposed twice for 2 hours: once to clean air and once to 0.3-ppm ozone. This concentration is comparable to that used in many previous controlled-human-exposure studies. Although higher than the Environmental Protection Agency National Ambient Air Quality Standards standard of 0.076 ppm and not seen in American cities, it is only slightly higher than peak hourly concentrations observed in heavily polluted cities such as Beijing and Mexico City.\(^8\)\(^9\) In addition, the subjects in this study were exposed to elevated levels of ozone for only 2 hours, in contrast to “real-life” situations that would involve much longer ozone exposures. Each exposure was separated by at least 2 weeks. During the 2-hour exposure, each subject alternated 15 minutes of rest with 15 minutes of exercise on a cycle ergometer. Minute ventilation was measured during each exposure, and exercise levels were adjusted to obtain a minute ventilation of 25 L/min · m\(^2\) body surface area. This exercise regimen is comparable to that done in most previous studies in which human volunteers were exposed to ozone under controlled conditions. It is meant to mimic people performing physical labor or exercising outdoors. The exposures were conducted at the Environmental Protection Agency Human Studies Facility on the campus of the University of North Carolina; the exposure chamber and generation of ozone have been described previously.\(^10\)\(^11\) Briefly, ozone was generated by a silent electric discharge method (model 502; Meckel, Bonn, Germany) and introduced into a chamber that was maintained at 22.6±1.0°C and 40–6.5% relative humidity. All exposures were conducted at the same time of day to avoid confounding by circadian variations.

**Measurements**

Spirometry (FVC, FEV\(_1\)) was performed immediately before exposure, immediately after exposure, and again the next morning as described earlier.\(^12\) Spirometry was performed with a Sensormedics Vmax 220 instrument and software (Sensormedics Corp, Yorba Linda, CA) according to American Thoracic Society guidelines.

Each subject underwent bronchoscopy with bronchoalveolar lavage 18 hours after the exposure as described previously.\(^13\) Cells were counted with a hemocytometer, and cell differentials were performed on cytocentrifuged slides stained with a modified Wright stain (Leukostat Solution, Fisher Scientific, Pittsburgh, PA). At least 200 cells per slide were counted.

Venous blood was sampled immediately before, 1 hour after, and 18 hours after each exposure. A differential blood count and blood lipid panel were performed by LabCorp (Burlington, NC). Commercially available ELISA kits were used to quantify levels of C-reactive protein (Alpcro Diagnostics, Windham, NH), dimer and von Willebrand factor (Diagnostica Stago, Parsippany, NY), tissue-type plasminogen activator (tPA) and plasminogen (Enzyme Research Laboratories, South Bend, IN), and plasminogen activator inhibitor-1 (PAI-1) (DakoCytomation, Carpentry, CA).

Continuous ambulatory ECGs (Holter) were collected for ~24 hours with a Mortara H12+ 12-lead ECG Recorder (Mortara Instrument Co, Milwaukee, WI). The digitally recorded ECGs were sampled at 1000 Hz, and a trained research nurse blinded to the exposure randomization manually edited the sequence of ECG complexes to ensure proper labeling of each QRS complex. Time-domain parameters (standard deviation of normal to normal and percentage of heart beats that are separated from neighboring beats by more than 50 milliseconds [pNN\(_{50}\)] were calculated over a 24-hour period starting at the beginning just before each exposure. Frequency-domain parameters were measured during three 30-minute periods (immediately before exposure and ~1 and 20 hours after the completion of exposure) while the subjects rested quietly in a darkened room. The final 5 minutes of recording during these resting periods was used for calculation of frequency domain and repolarization variables. High frequency (HF; 0.15–0.4 Hz) and low frequency (0.04–0.15 Hz) were calculated in milliseconds. Premature atrial contractions and premature ventricular contractions were calculated over the entire 24-hour monitoring period.

The effects on cardiac repolarization were assessed by measuring the QT interval corrected for heart rate (QTc). QTc was calculated from the raw Holter ECG data with the use of proprietary analysis software from Mortara, Inc, which corrects for heart rate by using a subject-specific QT/RR slope. The complexity of the QRS complex...
None of the participants reported any complaints or symptoms after exposure to air or ozone. Baseline values and postexposure and follow-up exposure values are shown in Table 1, as well as the postexposure/pre-exposure and follow-up/pre-exposure changes. Statistically significant ozone-induced changes are shown in Figure 1. Immediately after ozone exposure, we observed statistically significant postexposure/pre-exposure increases in blood levels of interleukin (IL)-1β and nearly significant increases in IL-8 and tumor necrosis factor-α compared with postexposure/pre-exposure changes after air exposure. There was an 85.3% increase in IL-8 (95% confidence interval [CI], 44.1–138.5), a 55.7% increase in IL-1β (95% CI, −5.1 to 152.9), and a 10.1% increase in

### Results

#### Effect of Ozone on Vascular Markers of Inflammation

None of the participants reported any complaints or symptoms after exposure to air or ozone. Baseline values and postexposure and follow-up exposure values are shown in Table 1. Table 1. Changes in Biomarkers of Inflammation

<table>
<thead>
<tr>
<th></th>
<th>Pre-Exposure Air</th>
<th>Postexposure Air</th>
<th>Follow-Up Air</th>
<th>Pre-Exposure O₃</th>
<th>Postexposure O₃</th>
<th>Follow-Up O₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood IL-1, pg/mL</td>
<td>0.29±0.09</td>
<td>0.25±0.06</td>
<td>0.21±0.03</td>
<td>0.21±0.05</td>
<td>0.24±0.05</td>
<td>0.35±0.08</td>
</tr>
<tr>
<td>Ratio</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood IL-6, pg/mL</td>
<td>2.25±0.26</td>
<td>2.18±0.25</td>
<td>2.18±0.23</td>
<td>2.12±0.31</td>
<td>2.13±0.27</td>
<td>2.09±0.27</td>
</tr>
<tr>
<td>Ratio</td>
<td>1.00±0.06</td>
<td>0.99±0.04</td>
<td></td>
<td></td>
<td>1.07±0.06</td>
<td>1.04±0.06</td>
</tr>
<tr>
<td>Blood IL-8, pg/mL</td>
<td>1.15±0.17</td>
<td>1.13±0.19</td>
<td>1.18±0.14</td>
<td>0.98±0.14</td>
<td>1.56±0.11</td>
<td>1.11±0.11</td>
</tr>
<tr>
<td>Ratio</td>
<td>1.05±0.10</td>
<td>1.16±0.10</td>
<td></td>
<td></td>
<td>2.04±0.27†</td>
<td>1.36±0.21</td>
</tr>
<tr>
<td>Blood TNF, pg/mL</td>
<td>4.83±0.26</td>
<td>4.32±0.23</td>
<td>5.27±0.37</td>
<td>4.64±0.35</td>
<td>4.49±0.27</td>
<td>4.95±0.31</td>
</tr>
<tr>
<td>Ratio</td>
<td>0.90±0.03</td>
<td>1.09±0.04</td>
<td></td>
<td></td>
<td>1.00±0.04†</td>
<td>1.11±0.06</td>
</tr>
<tr>
<td>Blood CRP, ng/mL</td>
<td>686±325</td>
<td>708±279</td>
<td>760±356</td>
<td>643±148</td>
<td>656±182</td>
<td>972±184</td>
</tr>
<tr>
<td>Ratio</td>
<td>1.10±0.08</td>
<td>1.26±0.26</td>
<td></td>
<td></td>
<td>1.15±0.11</td>
<td>2.62±0.76*</td>
</tr>
<tr>
<td>Blood PMNs, %</td>
<td>50.8±2.5</td>
<td>59.6±2.3</td>
<td>50.0±1.8</td>
<td>51.7±1.4</td>
<td>63.5±2.0</td>
<td>50.1±1.9</td>
</tr>
<tr>
<td>Ratio</td>
<td>1.18±0.38</td>
<td>0.99±0.02</td>
<td></td>
<td></td>
<td>1.22±0.04</td>
<td>0.97±0.03</td>
</tr>
<tr>
<td>BAL PMNs, %</td>
<td>1.9±0.34</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>9.6±1.3*</td>
</tr>
</tbody>
</table>

IL indicates interleukin; TNF, tumor necrosis factor; CRP, C-reactive protein; and PMN, polymorphonuclear neutrophil.

*P<0.025.
†P<0.010.

was also calculated with Mortara software and is defined as the ratio of the second eigenvalue to the first.

### Statistical Analysis

All end points measured 1 hour (postexposure) and 24 hours (follow-up) after exposure were divided by pre-exposure values and expressed as percent of the baseline (pre-exposure), with the exception of bronchoalveolar lavage, the 24-hour Holter time-domain data, and ectopic beats, which were collected only 24 hours after exposure. Normalization against pre-exposure values is regularly performed in controlled-exposure studies to account for day-to-day variability of baseline levels in subjects. By normalizing against pre-exposure values and comparing each person’s response to air exposure with the same person’s response to ozone exposure, we also control a number of other confounding parameters, eg, exercise, age, sex, medication use, and diurnal variation. The postexposure/pre-exposure and follow-up/pre-exposure values after both air and ozone exposure were used to calculate statistical significance.

Paired tests were used to assess differences between air and ozone, with each subject serving as his or her own control, minimizing variation in response among subjects. Because data were not distributed normally for some end points, to be consistent, all end points were log transformed before analysis. Changes after ozone exposure are expressed relative to changes after air exposure (percent of air exposure). A value of P<0.05 was considered significant, although it might also be appropriate to use a value of 0.025 for significance because data from 2 time points (postexposure and follow-up) are being analyzed. It is also recognized that because of the number of variables being analyzed in this study, some of the variables may appear significant owing to chance alone. In addition to paired t tests, the data were analyzed by mixed-effects models incorporating postexposure and follow-up findings independently or in the same model and by a bootstrap model. Because the findings from all 4 tests were consistent, only paired t test findings are shown in this article.

#### Figure 1. Ozone-induced Changes in Markers of Vascular Inflammation

For analysis, the pre-exposure values are subtracted from either the postexposure or follow-up values, and a paired t test is used to compare the differences after ozone exposure with the differences after air exposure. These differences are plotted as the mean and 95% confidence interval. For tumor necrosis factor (TNF), P=0.067; for interleukin (IL)-8, P<0.001; for IL-1 after exposure, P=0.008; for IL-1 follow-up, P<0.001; for C-reactive protein (CRP), P=0.023.
tumor necrosis factor-α (95% CI, −0.75 to 22.1). Some markers of inflammation were present as long as 24 hours after exposure. There was a 103.8% increase in follow-up/pre-exposure levels (95% CI, 53.8 to 69.2), which was nearly significant (P < 0.05).

Effect of Ozone on Vascular Markers of Thrombosis
In addition to inducing vascular inflammation, we determined whether exposure to ozone could cause a more prothrombotic environment. We measured blood concentrations of several proteins involved in the formation or dissolution of blood clots. Baseline values of vascular thrombosis and postexposure and follow-up exposure values are shown in Table 2, as well as the postexposure/pre-exposure and follow-up/pre-exposure changes. Statistically significant ozone-induced changes are shown in Figure 3. There was a 51.2% reduction in the postexposure/pre-exposure HF component of HRV immediately after ozone exposure (95% CI, −69.2 to −23.7) compared with postexposure/pre-exposure values after air exposure. There was a trend for decreased HF 24 hours after exposure, at which time there was a 32.8% decrease in HF (95% CI, −57.9 to −7.1). There was a small but significant ozone-induced 1.2% increase in the duration of the QT interval immediately after exposure when corrected for heart rate by the use of a subject-specific QT/RR slope (95% CI, 0.40–

Effect of Ozone on HRV and Repolarization
PM air pollution has been associated with altered autonomic nervous system control of the heart, as evidenced by changes in both HRV and repolarization. To determine whether ozone can affect the autonomic nervous system also, we measured changes in both the time and frequency domains for HRV and markers of repolarization. Baseline values of HRV and repolarization and postexposure and follow-up exposure values are shown in Table 3, as well as the postexposure/pre-exposure and follow-up/pre-exposure changes. Statistically significant ozone-induced changes are shown in Figure 3. There was a 51.2% reduction in the postexposure/pre-exposure HF component of HRV immediately after ozone exposure (95% CI, −69.2 to −23.7) compared with postexposure/pre-exposure values after air exposure. There was a trend for decreased HF 24 hours after exposure, at which time there was a 32.8% decrease in HF (95% CI, −57.9 to −7.1). There was a small but significant ozone-induced 1.2% increase in the duration of the QT interval immediately after exposure when corrected for heart rate by the use of a subject-specific QT/RR slope (95% CI, 0.40–
Exposure to ozone did not cause changes in other measures of spread of depolarization through the ventricular muscle. The ratio of the second eigenvalue to the first, represents the complexity, plotted as the mean and 95% confidence interval. For QRS with the differences after air exposure. These differences are tested is used to compare the differences after ozone exposure by either the postexposure or follow-up values, and a paired test is used to compare the differences after ozone exposure with the differences after air exposure. These differences are plotted as the mean and 95% confidence interval. For QRS complexity, \( P = 0.019 \); for QTc, \( P = 0.007 \); for postexposure high frequency (HF), \( P = 0.013 \); for follow-up HF, \( P = 0.002 \); for low frequency, \( P = 0.015 \); for heart rate, \( P = 0.002 \).

### Table 3. Changes in Holter Monitor Variables

<table>
<thead>
<tr>
<th></th>
<th>Pre-Exposure Air</th>
<th>Postexposure Air</th>
<th>Follow-Up Air</th>
<th>Pre-Exposure ( \text{O}_3 )</th>
<th>Postexposure ( \text{O}_3 )</th>
<th>Follow-Up ( \text{O}_3 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDNN, ms</td>
<td>91.2±3.6</td>
<td>91.2±3.6</td>
<td>91.2±3.6</td>
<td>88.2±4.6</td>
<td>88.2±4.6</td>
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</tr>
<tr>
<td>pNN50, %</td>
<td>26.2±2.5</td>
<td>26.2±2.5</td>
<td>26.2±2.5</td>
<td>24.2±2.5</td>
<td>24.2±2.5</td>
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</tr>
<tr>
<td>Number PAC</td>
<td>22.3±8.1</td>
<td>22.3±8.1</td>
<td>22.3±8.1</td>
<td>23.9±8.9</td>
<td>23.9±8.9</td>
<td>23.9±8.9</td>
</tr>
<tr>
<td>Number PVC</td>
<td>5.7±1.3</td>
<td>5.7±1.3</td>
<td>5.7±1.3</td>
<td>8.2±1.8</td>
<td>8.2±1.8</td>
<td>8.2±1.8</td>
</tr>
<tr>
<td>HF, ms</td>
<td>2630±424</td>
<td>2897±386</td>
<td>3484±429</td>
<td>3132±469</td>
<td>1833±399</td>
<td>2959±463</td>
</tr>
<tr>
<td>Ratio</td>
<td>1.37±0.14</td>
<td>2.04±0.41</td>
<td>0.73±0.14*</td>
<td>1.43±0.28†</td>
<td>2.65±0.78</td>
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<tr>
<td>LF, ms</td>
<td>2678±372</td>
<td>2576±390</td>
<td>2620±409</td>
<td>2857±493</td>
<td>1833±398</td>
<td>3218±554</td>
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<tr>
<td>Ratio</td>
<td>1.69±0.36</td>
<td>1.69±0.38</td>
<td>1.69±0.38</td>
<td>1.49±0.45</td>
<td>2.65±0.78</td>
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</tr>
<tr>
<td>HF/LF</td>
<td>1.06±0.16</td>
<td>0.75±0.10</td>
<td>0.76±0.11</td>
<td>1.05±0.14</td>
<td>0.72±0.11</td>
<td>1.10±0.27</td>
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<tr>
<td>Ratio</td>
<td>1.06±0.16</td>
<td>0.97±0.17</td>
<td>0.80±0.10</td>
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</tr>
<tr>
<td>QTc</td>
<td>412±5.2</td>
<td>407±4.6</td>
<td>410±5.2</td>
<td>408±4.4</td>
<td>408±4.9</td>
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</tr>
<tr>
<td>Ratio</td>
<td>0.95±0.003</td>
<td>0.99±0.008</td>
<td>1.00±0.003‡</td>
<td>1.00±0.017</td>
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<td>QTc complexity</td>
<td>0.31±0.03</td>
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<td>0.33±0.03</td>
<td>0.34±0.03</td>
<td>0.35±0.03</td>
<td>0.36±0.03</td>
</tr>
<tr>
<td>Ratio</td>
<td>1.04±0.03</td>
<td>1.06±0.02</td>
<td>1.03±0.02*</td>
<td>1.24±0.15</td>
<td>1.24±0.15</td>
<td>1.24±0.15</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>59.7±1.7</td>
<td>61.6±2.1</td>
<td>54.7±1.5</td>
<td>58.3±1.7</td>
<td>63.4±3.1</td>
<td>56.1±2.0</td>
</tr>
<tr>
<td>Ratio</td>
<td>1.04±0.03</td>
<td>0.92±0.02</td>
<td>1.09±0.04</td>
<td>0.99±0.06</td>
<td>0.99±0.06</td>
<td>0.99±0.06</td>
</tr>
</tbody>
</table>

SDNN indicates standard deviation of normal to normal; PAC, premature atrial contraction; PVC, premature ventricular contraction; HF, high frequency; and LF, low frequency.

\( *P<0.025 \)

\( †P<0.10 \)

Effect of Ozone on Lung Function and Pulmonary Inflammation

Although the primary emphasis in this study was on assessing cardiovascular changes caused by exposure to ozone, we also measured ozone-induced changes in FEV\(_1\) and bronchoalveolar lavage inflammatory cells to ascertain whether the study participants responded to ozone in a manner consistent with that reported in previous studies. Ozone-induced changes in FEV\(_1\) and bronchoalveolar lavage neutrophils are shown in Figure 4. Immediately after exposure to ozone, postexposure/pre-exposure FEV\(_1\) values increased relative to postexposure/pre-exposure air exposure values by 10.9% (95% CI, −6.5 to −15.2), which is consistent with changes reported in other studies in which participants were exposed to 0.2- to 0.4-ppm ozone.\(^{20}\) The difference between neutrophils found in bronchoalveolar lavage fluid 24 hours after ozone exposure relative to air exposure was 7.5% (95% CI, 3.7–15.3), which is consistent with what we and others have reported previously in participants exposed to 0.2- to 0.4-ppm ozone.\(^{21–23}\)

Discussion

We believe that the changes in ozone-induced vascular inflammation, fibrinolysis markers, and HRV detailed in this study provide the most compelling data to date of the potential for ozone to modulate the cardiovascular system. Previous attempts to study these outcomes in epidemiology studies have resulted in inconsistent results, with some showing positive associations between ozone and cardiovascular changes and others not. These inconsistencies are likely due to differences in exposure metrics and windows of exposure, different methodologies used to assess cardiovascular changes, and the difficulty of attributing associations to
ozone itself given its colocation across time and space with other pollutants (notably PM).4

In this study, we observed robust changes in several proinflammatory cytokines in the blood within an hour after exposure to ozone. One of them (IL-1β) persisted for at least 24 hours with no diminution. These increases in markers of inflammation reported in this study were both substantial and highly significant and thus not likely just due to chance. We also observed changes in C-reactive protein and a trend for increased concentration of neutrophils in the blood immediately after ozone exposure. In contrast, previous epidemiology studies reporting associations between ozone and inflammatory markers have been equivocal. A retrospective repeated measures analysis of 45 Canadian adults reported a positive association between ozone and IL-6, with the strongest effects observed for a 4-day moving average of ozone.29 Similarly, a study of 76 healthy individuals in Taiwan found associations between ozone and increased levels of C-reactive protein25; the strongest associations were with a 2-day averaging time. However, no association between ozone and C-reactive protein was observed in a repeated measures study of healthy individuals living in the Netherlands26 or in >3000 healthy individuals living in Israel.27 The causal nature of the findings from our controlled-exposure study supports the epidemiology studies that have reported positive associations between ozone and markers of inflammation.

We observed ozone-induced changes in several markers associated with fibrinolysis. A small increase in tPA was seen immediately after a 2-hour exposure to ozone. This serine protease catalyzes the conversion of plasminogen to plasmin, the major enzyme responsible for the breakdown of blood clots. There were more substantial and prolonged decreases in PAI-1. PAI-1 levels were decreased both immediately and 24 hours after ozone exposure. PAI-1, a serine protease inhibitor encoded by the SERPINE1 gene, is the principal inhibitor of tPA. The combination of an increase in tPA and a decrease in PAI-1 suggests the possibility that ozone might activate the fibrinolysis system, perhaps in response to fibrin deposition caused by ozone exposure, although other explanations are also possible. We have previously reported that activation of fibrinolysis pathways and increased levels of D-dimer (a fibrin breakdown product) are associated with exposure to PM air pollution in humans.28

We also show that ozone can mirror the established effects of PM and cause changes in HRV and cardiac repolarization. We observed a decrease in the HF component of HRV immediately after ozone exposure, which is consistent with findings in multiple PM studies (PM Integrated Science Assessment). A previous controlled-exposure study29 did not find significant ozone-induced changes in HRV. However, in this study, the participants were exposed to a smaller concentration of ozone (0.12 ppm) and did not exercise during exposure, which would have resulted in a smaller dose of ozone delivered to their airways. Controlled-exposure studies have shown altered HRV after combined exposure to particles and ozone.20,30

We also observed an increase in the duration of the QT interval immediately after exposure. A prolonged QT interval is a risk factor for ventricular tachyarrhythmias and sudden death. Some epidemiology studies have reported a positive association between ozone exposure and ventricular arrhythmias in people with implanted cardioverter-defibrillators31–33 and in nonsmoking adults 54 to 90 years of age.34 In contrast, no evidence of an association between ozone and tachyarrhythmia events was observed in a study of 518 implanted cardioverter-defibrillator patients.35 Associations between ozone exposure and decreased HRV (HF and low frequency) have been reported,36 although the same cohort did not show any evidence of an association between ozone and QTc.37 No associations were found between ozone and HRV in patients with congestive heart disease in Taiwan38 or France.39 A recent study reported associations between ozone exposure and increased heart rate, t-wave flattening, and increased T-wave complexity.40

The mechanism by which ozone can affect the cardiovascular system is not clear. Some studies have reported that PM can translocate from the lung to the vasculature where it could directly attack vascular cells or the heart. However, we believe it is an unlikely mechanism for ozone because ozone is a highly reactive molecule that is consumed within seconds of inhalation. Using ozone tagged with the heavy isotope of oxygen (18O), we found a small amount of heavy isotope in arterial atherosclerotic plaques can oxidize cholesterol.42 Given the reactivity of ozone, if it were to diffuse into
the circulatory system, the concentration in arteries would likely be higher than in veins, raising the intriguing possibility that if small quantities of ozone were to diffuse into the blood, they might contribute to oxidation of cholesterol found in the plaques. PM air pollution has recently been reported to be able to oxidize cholesterol, presumably by translocation of particles from the lung into the circulatory system.46,47 A second proposed mechanism has been the spillover of pollutant-induced mediators from the lung into the blood, which could interact with vascular or cardiac cells. Ozone is known to cause robust increases in a number of pulmonary markers, including proinflammatory cytokines, eicosanoids, tPA, and fibrinogen.21,44,45 These increases are much more substantial than those observed after exposure of humans to PM, and they are initiated within 1 hour after a 2-hour exposure and can persist for at least 24 hours.46,47 A third mechanism is moduation of the autonomic nervous system via nerve endings in the lung, which then affects cardiac and vascular function. Ozone-induced lung-function changes are also known to be mediated by C nerve fibers in the lung of humans48 via the selective stimulation of transient receptor potential cation channel, subfamily A, member 1 ion channels,29 and it is likely that the autonomic nervous system HRV and repolarization changes reported here are also mediated via nerve fibers that terminate in the lung. Recent rodent studies show a significant role for autonomic modulation of HRV and arrhythmia after exposure to ozone. These responses appear to involve both central nervous system and target receptors (transient receptor potential cation channel, subfamily A, member 1).50

Conclusions
This study shows that exposure of healthy young adults to ozone causes an increase in vascular markers of inflammation, changes in fibrinolytic markers that could potentially impair fibrinolysis, and changes in autonomic control of heart rate. These changes could potentially put a susceptible individual at risk for an adverse clinical event and thus provide biological plausibility to the epidemiology studies that have reported associations between ozone exposure and mortality/morbidity.

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Disclosures
None.

References


**CLINICAL PERSPECTIVE**

Many epidemiology studies report an association between airborne particulate matter and increased cardiovascular morbidity and mortality in susceptible populations. More recent epidemiology studies have also reported a similar association with ozone. However, the pathophysiological pathways that underlie adverse clinical events after ozone exposure are not well understood. Ozone is a highly reactive oxidant, and in contrast to inhaled particulate matter, which may leave the lung and enter the bloodstream where it could directly attack vascular or cardiac tissue, ozone is not believed to leave the lung. Using a randomized crossover design, we exposed 23 young healthy individuals to clean air and to 0.3-ppm ozone for 2 hours. Ozone exposure resulted in extrapulmonary effects, including a decrease in cardiac parasympathetic tone and plasminogen activator inhibitor-1 and an increase in measures of systemic inflammation and cardiac repolarization. The study confirmed previous reports of ozone-induced decreases in lung function and increased pulmonary inflammation. The ozone-induced changes reported here are similar to changes reported in humans exposed to particulate matter, suggesting that both pollutants may cause effects by the same pathways. This report provides some insight into potential mechanisms that might contribute to the risk for adverse clinical cardiovascular effects in susceptible individuals. It also supports improving environmental health literacy among physicians and counseling patients at highest risk from air pollutants to take measures to protect themselves such as use of the local Air Quality Index.
Controlled Exposure of Healthy Young Volunteers to Ozone Causes Cardiovascular Effects
Robert B. Devlin, Kelly E. Duncan, Melanie Jardim, Michael T. Schmitt, Ana G. Rappold and David Diaz-Sanchez

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