Bronchial reactivity of healthy subjects: 18–20 h postexposure to ozone

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Foster, W. Michael, Robert H. Brown, Kristin Macri, and Clifford S. Mitchell Bronchial reactivity of healthy subjects: 18–20 h postexposure to ozone. J Appl Physiol 89: 1804–1810, 2000.—Exposure of humans to ambient levels of ozone (O₃) causes inflammatory changes within lung tissues. These changes have been reported for the “initial” (1- to 3-h) and “late” (18- to 20-h) postexposure periods. We hypothesized that at the late period, when protein and cellular markers of inflammation at the airway surface remain abnormal and the integrity of the epithelial barrier is compromised, bronchial reactivity would be increased. To test this, we measured airway responsiveness to cumulative doses of methacholine (MCh) aerosol in healthy subjects 19 ± 1 h after a single exposure to O₃ (130 min at ambient levels between 120 and 240 parts/billion and alternate periods of rest and moderate exercise) or filtered air. Exposures were conducted at two temperatures: mild (22°C) and moderate (30°C). At the late period, bronchial reactivity to MCh increased, i.e., interpolated dose of MCh leading to a 50% fall in specific airway conductance (PC₅₀) was less after O₃ than after filtered air. PC₅₀ for O₃ at 22°C was 27 mg/ml (20% less than the PC₅₀ after filtered air), and for O₃ at 30°C it was 19 mg/ml (70% less than the PC₅₀ after filtered air). The forced expiratory volume in 1 s (FEV₁) at the late time point after O₃ was slightly but significantly reduced (2.3%) from the preexposure level. There was no relationship found between the functional changes observed early after exposure to O₃ and subsequent changes in bronchial reactivity or FEV₁ at the late time point. These results suggest that bronchial reactivity is significantly altered ~1 day after O₃; this injury may contribute to the respiratory morbidity that is observed 1–2 days after an episode of ambient air pollution.

specific airway conductance; methacholine

OZONE (O₃), a highly reactive gas, is a natural constituent of the upper atmosphere and is a major component of ambient smog formed by the reaction of primary air pollutants (hydrocarbons and oxides of nitrogen) in the presence of sunlight. An edemagenic lung irritant, O₃ affects the conducting airways and alveolar regions. Toxicity of O₃ to epithelial surfaces is primarily attributed to reactive oxygen species and ozonation of unsaturated fatty acids present in lung lining fluids (29). Early (3-h) and late (18- to 20-h) responses in the human after whole lung exposure to O₃ include an influx of protein, inflammatory cells, and mediators into airway and alveolar surface fluids (2, 4, 35). The presence of these cellular and biochemical markers of injury at the epithelial surface and the increased diffusivity of small molecules within the submucosa postexposure (14) have led to speculation that exposure to O₃ modulates bronchial smooth muscle tone and responsiveness to nonspecific challenge (24).

Airway hyperresponsiveness is present in all humans with asthma, at least when symptomatic. The long-term effect(s) of increased airway responsiveness are unknown, although it may be a risk factor for the development of chronic obstructive lung disease (34). Increased airway smooth muscle tone can lead to nonhomogeneous ventilation and limit the maximum oxygenation of pulmonary blood; both of these physiological responses may be exacerbated by O₃ (13, 33). The extent to which O₃ affects airway responsiveness is unclear. Whereas an ambient level of O₃ does not provoke airway hyperresponsiveness either immediately or 24 h postexposure (26), increases in airway responsiveness have been observed in the immediate postexposure period when O₃ has been combined with heavy exercise (22) or after high effective concentrations (400–600 parts/billion (ppb)) (21, 25, 32). The duration of changes in airway reactivity after exposure to high concentrations (≥400 ppb) of O₃ has not been determined. A recent study of asthmatic and healthy subjects exposed to 400 ppb O₃ has reported that airway hyperreactivity was present at a time point at least 12 h postexposure (20). Moreover, an earlier investigation reported that, even with high levels of O₃, provocative responses to nonspecific aerosol challenge return to baseline responsivity (preexposure) by 24 h (21). However, in several chamber studies, inflammation is present in airway and pulmonary tissues 24 h postexposure (2, 35), and many epidemiological studies have documented that a lag time of 1–2 days follows acute episodes of oxidant air pollution before morbidity effects are observed (28, 36).

The purpose of our study was to test the hypothesis that ambient levels of O₃ when combined with ambient temperature conditions can induce a state of airway

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hyperresponsiveness ~1 day postexposure. As a first step, we used a crossover design to expose healthy, nonasthmatic subjects to filtered air (FA) and O₃ at mild- and moderate-temperature conditions. Approximately 1 day after exposure, we evaluated nonspecific hyperresponsiveness of the subjects to cumulative challenge doses of methacholine (MCh) aerosol.

METHODS

Subject description and characterization. Nine healthy subjects (4 women and 5 men) were recruited for the study. All were nonsmokers, without a history of lung disease, and not receiving medications for any other disease. Age, anthropometric characteristics, and spirometric lung function values of the subjects are listed in Table 1. The subjects had a mean age of 26 ± 2 (SD) yr and were free of respiratory infection at the time of the evaluations. Subjects’ mean values of the forced vital capacity (FVC), the forced expiratory volume in 1 s (FEV₁), and the midmaximal expiratory flow rates (FEF 25–75) were >92 ± 11% (SD) of predicted. Consent was obtained from the subjects before admission to the study, and the research had the approval of the University Human Research Review Board.

Experimental protocol. An initial (baseline) evaluation of pulmonary function and the treadmill (model Q55, Quinton Instruments, Seattle, WA) exercise required to attain cumulative ventilation per minute during exercise that was approximately equal to eight times the volume of the FVC was conducted on each subject. On 4 additional study days, the subjects returned to the laboratory at an appointed time and were exposed for a 130-min period to either FA or O₃ in a walk-in chamber facility (14.2 m³). For each exposure (FA and O₃), chamber air was held at 45–55% relative humidity and at a mean temperature of either 22 or 30°C. Exposure of a given subject commenced at the same time of the day; the order for crossover of the exposures (FA at 22°C, FA at 30°C, O₃ at 22°C, O₃ at 30°C) was randomized with a minimum 10-day interval between the reexposure study days. Spirometry (at least 3 determinations) was measured before exposure, at midexposure, and at 10 min postexposure, and measurements of specific airway conductance (sGaw) and thoracic gas volume were accomplished at the before-exposure and 10-min postexposure assessment times by using a body plethysmographic method (Sensor Medics, Anaheim, CA) (7); spirometric and plethysmographic measures were repeated 18–20 h (~1 day) postexposure. Immediately after these functional measures at ~1 day postexposure, the measurement of airway hyperresponsiveness to cumulative MCh aerosol challenge was assessed.

Table 1. Age, anthropometric, and lung function characteristics of subjects

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Age, yr</th>
<th>Height, in.</th>
<th>Weight, lb.</th>
<th>Gender</th>
<th>FVC, liters</th>
<th>FEV₁, liters</th>
<th>FEF 25–75, l/s</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>32</td>
<td>72</td>
<td>178</td>
<td>M</td>
<td>5.58</td>
<td>4.58</td>
<td>4.20</td>
</tr>
<tr>
<td>2</td>
<td>32</td>
<td>64</td>
<td>132</td>
<td>F</td>
<td>3.64</td>
<td>3.15</td>
<td>3.74</td>
</tr>
<tr>
<td>3</td>
<td>26</td>
<td>65.5</td>
<td>128</td>
<td>F</td>
<td>3.52</td>
<td>3.12</td>
<td>3.46</td>
</tr>
<tr>
<td>4</td>
<td>26</td>
<td>75</td>
<td>179</td>
<td>M</td>
<td>5.31</td>
<td>4.87</td>
<td>6.42</td>
</tr>
<tr>
<td>5</td>
<td>24</td>
<td>68</td>
<td>165</td>
<td>M</td>
<td>5.63</td>
<td>4.43</td>
<td>3.76</td>
</tr>
<tr>
<td>6</td>
<td>24</td>
<td>66</td>
<td>165</td>
<td>M</td>
<td>5.27</td>
<td>4.42</td>
<td>4.92</td>
</tr>
<tr>
<td>7</td>
<td>27</td>
<td>67</td>
<td>151</td>
<td>F</td>
<td>3.56</td>
<td>3.31</td>
<td>3.90</td>
</tr>
<tr>
<td>8</td>
<td>26</td>
<td>74.5</td>
<td>189</td>
<td>M</td>
<td>6.02</td>
<td>4.68</td>
<td>4.22</td>
</tr>
<tr>
<td>9</td>
<td>25</td>
<td>67</td>
<td>149</td>
<td>F</td>
<td>4.21</td>
<td>3.58</td>
<td>3.78</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>26.9 ± 1.0</td>
<td>68.8 ± 1.3</td>
<td>160 ± 7.1</td>
<td></td>
<td>4.75 ± 0.34</td>
<td>4.02 ± 0.24</td>
<td>4.37 ± 0.29</td>
</tr>
</tbody>
</table>

FVC, forced vital capacity; FEV₁, forced expiratory volume in 1 s; FEF 25–75, midmaximal expiratory flow rate; M, male; F, female.
bias) powered with FA at 30 psi; nebulization of MCh for each challenge breath commenced after the subjects inspired a 150-ml volume and lasted for 0.6 s. The starting dose level of MCh was 2.5 mg/ml, which was then increased on each subsequent challenge to a maximal level of 22.5 mg/ml. After the fifth aerosol breath at each MCh dose, subjects relaxed for 3 min and then had spirometric and plethysmographic responses measured for each cumulative MCh dose inhaled. The subjects did not demonstrate changes in spirometric flows, and therefore airway responsiveness was determined as the cumulative MCh dose leading to a decrease in the sGaw of at least 50% from the value of the sGaw observed after the saline challenge.

Statistical analysis. Pulmonary function volume measurements were corrected to body temperature and pressure of gas saturated with water vapor (BTPS), and the trials with the highest FVC and FEV1 for preexposure, midexposure, end exposure, and ~1 day postexposure were utilized for statistical analysis. Means ± SE were calculated for spirometric function and plethysmographic measures with standard statistical methods. On the basis of the percent decrease from the reference value (0.9% saline) of the sGaw after each succeeding concentration of MCh, the following equation (1) was used to calculate the interpolated PC50 (aerosol concentration of MCh leading to a 50% fall in the sGaw)

$$PC_{50} = \text{antilog} \left[ \log C_1 + \frac{(\log C_2 - \log C_1)(50 - R_1)}{R_2 - R_1} \right]$$

where C1 is the second-to-last MCh concentration, C2 is the final MCh concentration (concentration leading to a 50% or greater fall in sGaw), R1 is the percent fall in sGaw after C1, and R2 is the percent fall in sGaw after C2. Treatment (FA and O3) effects on airway responses and responsivity to MCh aerosol challenge doses were compared with analysis of variate for repeated measures and a Newman-Keuls post hoc test for significance of the differences. A paired t-test was used to compare the differences between the means of the calculated PC50 doses for the respective exposure conditions. A P value < 0.05 was considered significant.

RESULTS

Measures of functional response. By design, during the exercise periods of each chamber exposure, a targeted minute ventilation was achieved. The mean minute ventilation attained by the subjects for the exercise periods are presented in Fig. 1. Mean minute ventilations ranged between 36.40 ± 0.5 (SE) and 38.7 ± 1.0 l/min and were not significantly different between exposures. In general, during exposures to O3, the subjects reduced the depth of the tidal volume with a compensatory increase in the frequency of respiration, i.e., mean tidal volume during exercise periods for exposures to O3 at 22 and 30°C were 1.84 and 1.72 liters compared with the mean tidal volumes of 2.02 and 1.89 liters for exposures to FA at the respective temperatures. The mean changes (before MCh challenge) in pulmonary function of the subjects after exposures to O3 and FA for the different temperature conditions are presented in Fig. 2. For the FVC and FEV1, the responses at midexposure and end exposure and at ~1 day postexposure are compared with the functional values observed preexposure. Reductions in the FVC and the FEV1 were significant at midexposure and end exposure to O3 (mean decrements were on the order of 2–7%) for both ambient temperature conditions (22 and 30°C) compared with responses after FA exposure at the respective temperatures. The FEV1 remained reduced at the 1-day-postexposure time point compared with the preexposure value; although the change was small, i.e., a mean decrease of 2.3% from the preexposure level, this reduction was significant. The FVC (Fig. 2) had recovered to baseline by the 1-day-postexposure time point.

Changes in the sGaw are presented in Fig. 3; only the change in the sGaw (mean fall of 13.4%) at the end-exposure time point for exposure to O3 at an ambient temperature of temperature of 30°C was significant compared with the baseline sGaw and response to FA exposure at 30°C. There was a tendency for the sGaw to be elevated ~1 day postexposure, i.e., improved compared with the preexposure level; however, mean values of the sGaw were not significantly different from each other or from baseline values.

Measures of response to MCh challenge. At the 1-day-postexposure time point, the change in sGaw of the subjects to cumulative concentrations of MCh aerosol was a sensitive index for the assessment of airway responses. Measures of the FVC and the FEV1 were not sensitive in our healthy subjects for assessing airway responsiveness to MCh challenge. Mean values of the change in the sGaw are compared in Fig. 4 for each exposure condition, and responses to succeeding doses of MCh are expressed as a percent change from the response to diluent, i.e., 0.9% saline. At the starting point of each MCh challenge, the sGaw was not significantly different from the preexposure value of the sGaw (see Fig. 3). Compared with the responses after exposure to FA, the airways were clearly more responsive to MCh after exposure to O3. sGaw at the highest MCh challenge concentration (22.5 mg/ml) for O3 exposures and ambient temperature conditions of 22 and
30°C were significantly reduced compared with the corresponding FA exposures. There was the tendency for the sGaw response to MCh to be enhanced when O₃ was combined with the warmer exposure condition.

The interpolated PC₅₀ values of the subjects are listed in Table 2 for each exposure condition. The mean PC₅₀ calculated for exposures at 22°C was 20% less after O₃ than FA (27.4 vs. 34.1 mg/ml; P < 0.05), and for exposures at 30°C the mean PC₅₀ was further decreased, i.e., 70% less after O₃ than FA (19.3 vs. 66.2 mg/ml; P < 0.01).

**DISCUSSION**

This study in healthy adult nonsmokers demonstrates that airway hyperresponsiveness can be induced by O₃ exposure and measured 1 day postexposure. Nonspecific aerosol challenge clearly demonstrated that the airways were hyperresponsive 1 day after exposure to O₃ compared with FA (P < 0.01). There was a tendency for the warmer exposure condition, i.e., 30 vs. 22°C, to enhance this response. As expected, mean spirometric indexes of airway function (e.g., FEV₁) were decreased significantly during and acutely after exposure to O₃ (P < 0.05), and on average the FEV₁ at 1 day postexposure had almost completely recovered (97.7% of the preexposure value; P < 0.05).

This is the first human study to characterize an O₃-induced increase in airway responsiveness ~1 day postexposure. The subjects were exposed for 130 min to a ramped O₃ concentration (mean level of 175 ppb) at two ambient temperatures (22 and 30°C) and with a mild level of treadmill exercise. An additional functional index that was abnormal ~1 day postexposure was the FEV₁, and although the response data were not included in the RESULTS, the FEF₂₅–₇₅ was similarly slightly and significantly reduced (mean decrease of 4.0%; P < 0.05) below the baseline values of the FEF₂₅–₇₅ recorded ~24 h earlier, preexposure to O₃.

Our laboratory previously noted the failure of spirometric indexes of expiratory airflow to fully recover ~1 day postexposure.
day postexposure to O₃ (15, 16). In the present study, O₃-induced changes in sGaw during cumulative MCh aerosol challenge did not correlate with delayed recovery in the FEV₁ or FEF₂₅₋₇₅. It has been suggested that changes in lung function during and immediately post-exposure to O₃ are related to irritant and neural mechanisms (19, 27), whereas the delayed changes in the FEV₁ and FEF₂₅₋₇₅ 1 day postexposure may represent epithelial injury, receptor dysfunction, and inflammation at distal airway sites (14, 25).

Increases in nonspecific airway responsiveness have been observed acutely postexposure (immediately to 12 h after exposure) in healthy subjects exposed to O₃. These changes in airway reactivity have been reported for both low ambient (120–240 ppb) and high effective (400–600 ppb) chamber concentrations of O₃ (5, 21, 32). The acute development of airway hyperreactivity may be vagally mediated, and the responsiveness can be reversed with atropine premedication (18, 21). Investigations have utilized single- or multiple-exposure plans (9, 22); however, assessments of hyperreactivity were not usually conducted at later time points (i.e., 1 day postexposure) when O₃-induced inflammation of the airways and lung parenchyma have been well characterized (11). In one study of healthy subjects, an O₃ concentration of 200 ppb did not provoke airway responsiveness either immediately or 1 day postexposure (26), although hyperresponsive airways have been reported after exposure to high concentrations (400–600 ppb) of O₃ (18, 32). The duration of changes in airway reactivity after exposure to high effective concentrations (≥400 ppb) of O₃ is also not certain. Airway hyperreactivity was found at a time point at least 12 h postexposure in asthmatic and healthy subjects exposed to 400 ppb O₃ (20); however, an earlier investigation suggested that, even at high concentrations of O₃, airway responsiveness to either histamine or MCh aerosol returns to baseline responsivity (preexposure) within 24 h (18). Our results in healthy subjects demonstrate that airway hyperresponsiveness to MCh was clearly present 1 day postexposure. Airway conduc-

tance measures in our subjects had returned to baseline values at the start of the MCh challenge. Airway responsiveness to cumulative MCh challenge was significantly increased by exposure to O₃ at both the 23 or 25°C exposure temperatures, and there was a tendency for O₃ in combination with warmer exposure conditions to induce a greater degree of airway responsiveness. Similar to other phenotypic markers of O₃ exposure (2, 16, 31), a range in sensitivity of the airway response to O₃ was present from no change in airway reactivity (subject 9) to a >50% decrease of the PC₅₀ (subject 4).

Table 2. Interpolated provocative dose decreasing sGaw by 50% (PC₅₀)

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Filtered Air-22°C</th>
<th>Filtered Air-30°C</th>
<th>Ozone-22°C</th>
<th>Ozone-30°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15</td>
<td>9</td>
<td>43</td>
<td>11</td>
</tr>
<tr>
<td>2</td>
<td>43</td>
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<td>38</td>
</tr>
<tr>
<td>9</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

Mean ± SE 34.1 ± 11.6 27.4 ± 9.4* 66.2 ± 15.1 19.3 ± 4.1†

Values are given in mg/ml. Interpolated provocative dose of methacholine leading to 50% decrease in the specific airway conductance (sGaw) (PC₅₀) was calculated by using equation given in METHODS. *Mean dose significantly less than the mean dose for filtered-air exposure at 22°C, P < 0.05. †Mean dose significantly less than the mean dose for filtered-air exposure at 30°C, P < 0.05.
and epithelial cells) (11). Because O₃ does not penetrate cells, it can lead to several pulmonary and non-pulmonary events, and a cascade mechanism has been proposed to account for its toxicity (29). For example, O₃ reacts with unsaturated fatty acids at the air-tissue barrier to form lipid ozonation products that include aldehydes, hydroxyhydroperoxides, and Creigee ozonide; lipid by-products in turn can activate epithelial membrane lipases and release additional mediators onto the airway surface. Support for this sequence includes increased levels of mediators such as PGF₂α, proinflammatory cytokines, and reactive oxygen intermediates in airway fluids sampled ~24 h postexposure to O₃ (11). These by-products and mediators may also upregulate transcription factors such as nuclear factor-κB and proinflammatory genes (6) as well as modulate airway reactivity to nonspecific stimuli (24).

In addition, desquamation and disruption of airway epithelial membranes by O₃ would increase accessibility of MCh and other inhaled irritants and cellular mediators to epithelial sensory nerves and the bronchial musculature (8). In support of this mechanism, our laboratory previously reported that ~1 day after exposure of healthy subjects to O₃ there is a loss of epithelial integrity and an increase in airway permeability (14). Other than a temporal association of the two responses (changes in airway responsiveness and epithelial permeability) being present at the same time point postexposure to O₃, we do not have additional evidence linking the duration of airway responsiveness to the loss of epithelial integrity. In a large-animal model, however, we have found that O₃-induced changes in airway permeability can persist for up to 7 days postexposure (12). In addition, there is increasing evidence in animal models that O₃-induced airway hyperreactivity may be enhanced by hotter exposure conditions and pattern of breathing, i.e., rapid and shallow breaths, neuronal M₂ muscarinic receptor function is prevented by cyclo-

In summary, our study has shown that an increase in airway reactivity to nonspecific stimulation occurs ~1 day postexposure to O₃ in healthy subjects. There was a tendency for the airway responses to MCh to be enhanced by an elevation in temperature during exposure, and although significant, but slight, decrements in indexes of forced expiratory flow were also apparent ~1 day postexposure to O₃, these spirometric changes were not associated with alterations in airway hyper-responsiveness. Effects of inhalable irritants on the pattern of breathing, i.e., rapid and shallow breaths, may be enhanced by hotter exposure conditions and shift regional deposition of O₃ into dependent airways (23). Because frequently O₃ generation occurs during periods of increasing ambient temperature (30), our study suggests that O₃-induced airway responsiveness may be exacerbated during summer heat waves and causal to an increase in respiratory morbidity during episodes of oxidant air pollution.

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