



STATEMENT

Synopsis of Research Report 192, Part I

HEALTH
EFFECTS
INSTITUTE

Health Effects of Low-Level Ozone Exposure in Older Volunteers

INTRODUCTION

Ozone has been associated with adverse health effects in children and adults. Its effects on the respiratory system are well established and include worsening of asthma symptoms (acute effects), increases in deaths and hospital admissions for respiratory illnesses such as chronic obstructive pulmonary disease and asthma (acute and chronic effects), reduced lung growth, and higher risk of developing asthma (chronic effects). More recent epidemiological studies have reported that short-term exposure to ozone is associated with adverse cardiovascular outcomes, including an increased risk of cardiovascular mortality. The effects of ozone on the respiratory system are relatively well characterized, but its effects on the cardiovascular system are not. In view of the epidemiological findings, it has been suggested that ozone may lead to adverse cardiovascular health effects at concentrations at or below the current U.S. ambient air quality standard. Thus, research is needed to investigate the cardiovascular effects of ozone, particularly at concentrations near those of present-day ambient levels.

Ozone is an oxidant gas that readily reacts with other molecules. After inhalation, ozone reacts with constituents of the lung lining fluid to generate reactive oxygen species that can cause localized oxidative stress in the lung, leading to lung irritation. With repeated exposure, oxidative stress may lead to lung injury and chronic lung disease. Ozone may have effects on the cardiovascular and other organ systems through systemic inflammation, oxidative stress, or changes in activity of the autonomic nervous system, which could lead to changes in heart rhythm, endothelial dysfunction, constriction of arteries, and blood clotting.

In 2010, HEI issued Request for Applications 10-1, *Cardiovascular Effects of Exposure to Low Levels of Ozone in the Presence or Absence of Other Ambient Pollutants*, to solicit responses from clinical

research centers that were equipped to conduct human exposure studies, with the goal of creating a multicenter ozone study. Three centers, led by Dr. John Balmes at the University of California–San Francisco, Dr. Philip Bromberg at the University of

What This Study Adds

- Ozone exposure has been associated with acute and chronic respiratory effects, and there is some evidence of cardiovascular effects. However, it is unclear whether ozone has short-term cardiovascular effects at present-day ambient levels.
- This study measured a large number of cardiovascular and respiratory endpoints in 87 healthy, older participants who were exposed to 0, 70, or 120 parts per billion ozone for 3 hours while exercising moderately.
- There was no convincing evidence that ozone exposure in this large study of older, healthy adults affected the primary cardiovascular endpoints identified by the investigators. The observed lack of cardiovascular effects may not be generalizable to the overall adult population, which may include people who are less healthy and who are exposed to multiple pollutants.
- The study found moderate effects on lung function and on two markers of lung injury and inflammation in these healthy, older adults (a population that had not often been studied in the past), and provides confirmation of ozone effects on the lung at concentrations similar to the current air quality standard.

This Statement, prepared by the Health Effects Institute, summarizes a research project funded by HEI and conducted by Drs. Mark W. Frampton, Pulmonary & Critical Care, University of Rochester Medical Center, Rochester, NY; John R. Balmes, University of California–San Francisco; Philip A. Bromberg of the University of North Carolina–Chapel Hill; and Paul Stark of the New England Research Institute, Watertown, Massachusetts, and their colleagues. The complete report, *Multicenter Ozone Study in oldER Subjects (MOSES): Part 1. Effects of Exposure to Low Concentrations of Ozone on Respiratory and Cardiovascular Outcomes* (© 2017 Health Effects Institute), can be obtained from HEI or our website (see last page).

MOSES 192, Pt 1

Research Report 192, Part I

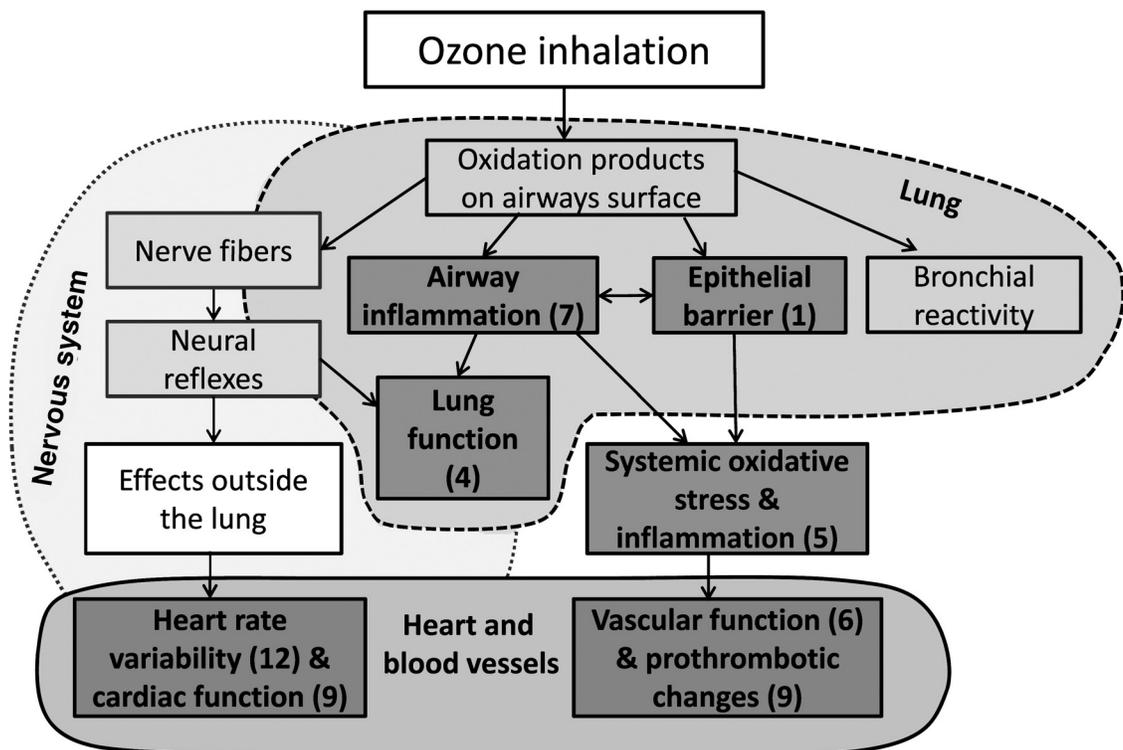
North Carolina—Chapel Hill, and Dr. Mark Frampton at the University of Rochester Medical Center, New York, were selected to participate in the study, which was named the Multicenter Ozone Study in older Subjects (MOSES). In addition, the New England Research Institute was selected through a 2010 *Request for Qualifications for a Data Analysis Center* to serve as the data coordinating center for the study. HEI formed a special MOSES Oversight Committee to provide input during the study design and research phases.

APPROACH

The MOSES project was funded to study the effects of short-term exposure to ozone on the cardiovascular and respiratory systems in older participants, a population presumed to be more susceptible to its effects. The study focused on low ozone concentrations (70 and 120 parts per billion [ppb]), relevant to those observed in ambient air in the United States. The three MOSES teams, with input from HEI, developed a common protocol for exposing human volunteers to ozone. Each center planned to recruit and test about 30 participants for a total of 90. Exposures took place

from mid-2012 to mid-2015. Each participant was invited to a screening visit, a training visit, and three exposure sessions (randomized at 0, 70, and 120 ppb ozone). Exposures lasted 3 hours, during which the participants exercised on a stationary bicycle, alternating 15 minutes of exercise with 15 minutes of rest. Participants stayed at a hotel the night before testing to minimize variability in exposure to ambient air pollutants and were evaluated the day before, during, and up to 22 hours after exposure.

The investigators measured a large suite of endpoints, including changes in autonomic nervous system function, heart rhythm, blood pressure, and pulmonary function, as well as markers of endothelial function, thrombosis, lung injury, and both systemic and lung inflammation (see Statement Figure). They specified in advance a key group of cardiovascular endpoints as primary; all other endpoints were secondary. Most outcomes were assessed at designated central laboratories that handled samples or electrocardiographic recordings from all three clinical centers in order to standardize outcome assessment. Study participants were also genotyped for glutathione S-transferase mu 1 (GSTM1),



Statement Figure. Possible pathways by which ozone may cause adverse health effects. Pathways evaluated in MOSES are shown in bold-face; the number of endpoints evaluated is shown in brackets. Adapted from Investigators' Report, Figure 1.

Research Report 192, Part I

a gene involved in antioxidant defenses. Individuals who lack the GSTM1 gene may be at increased risk for acute health effects.

A statistical analysis plan was developed and power calculations performed with input from investigators at the three clinical centers and the HEI MOSES Oversight Committee. Results were analyzed by mixed-effects linear models, adjusting for the three centers and multiple time points, and presented as the difference between pre-exposure and post-exposure values. The investigators tested whether the effects of ozone on each endpoint varied by subgroups defined by sex, age, or GSTM1 status. The statistical significance threshold was set at $P < 0.01$ to reduce concerns over multiple comparisons.

At the request of the HEI Research Committee, the investigators measured each participant's exposure to ozone and nitrogen dioxide using a personal sampler for 72 hours before the pre-exposure visit. They also collected air quality data for ozone, fine particulate matter, nitrogen dioxide, sulfur dioxide, and carbon monoxide from central monitors closest to each clinical center. A forthcoming report (Multicenter Ozone Study in oldEr Subjects, Part 2) will describe analyses that include the pre-exposure pollutant data, as well as several sets of sensitivity analyses conducted by the investigators.

KEY RESULTS

The three centers successfully recruited and tested 87 participants (ages 55–70 years) who completed all visits. Analyses of the primary cardiovascular endpoints showed no statistically significant effects of ozone exposure at 70 or 120 ppb on autonomic nervous system function, cardiac electrical repolarization, or cardiac arrhythmia. In addition, ozone exposure did not lead to statistically significant changes in oxidative stress or in markers of systemic inflammation, vascular function, or prothrombotic status. The only changes associated with ozone exposure seen in cardiovascular endpoints were an increase in the secondary endpoint plasma endothelin-1 (a marker of vascular function) and a decrease in nitrotyrosine (a marker of oxidative stress) after exposure to 120 ppb, but not 70 ppb, ozone.

On the other hand, the MOSES study confirmed that ozone has effects on the respiratory system even at these low concentrations, even though cardiac effects were not observed. In these older volunteers, moderate exercise during clean air exposure (0 ppb) led to an increased forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV₁) 15 minutes after exposure compared with pre-exposure values, and

they remained significantly higher after 22 hours. However, these improvements in lung function were attenuated after ozone exposure in a dose–response manner at 70 and 120 ppb. In addition, ozone exposure at 120 ppb significantly increased the percentage of polymorphonuclear leukocytes (a marker of lung inflammation; also referred to as “neutrophils”) in sputum as well as of club cell 16 (a marker of airway epithelial cell injury) in blood 22 hours later, compared with clean air exposure. In contrast, changes in sputum concentrations of the inflammatory markers interleukin-6, interleukin-8, and tumor necrosis factor-alpha were not statistically significant. There was no evidence of statistically significant interactions between sex, age, or GSTM1 status and the observed changes in lung function, sputum polymorphonuclear leukocytes, or plasma club cell 16 after ozone exposure.

EVALUATION

In its independent review of the study, a specially convened HEI MOSES Review Panel commended the investigators for a well-designed and executed study. A key strength of the study was the double-blind crossover design with controlled exposures at three concentrations. The Review Panel also noted that the number of participants in the MOSES study was considerably larger than in previous human exposure studies conducted to date and thought the study had sufficient statistical power to detect meaningful changes in the primary outcomes. The study efficiently collected information on a comprehensive array of cardiovascular endpoints, probing a variety of potential mechanistic or pathophysiological pathways, as well as several respiratory endpoints.

The Panel agreed with the investigators' conclusions that ozone exposure at 70 or 120 ppb for three hours did not lead to detectable changes in cardiovascular endpoints in this healthy group of older participants. Changes were observed in only two of the many cardiovascular endpoints: an increase in endothelin-1 and a decrease in nitrotyrosine. The nitrotyrosine changes were in the opposite direction of what would be hypothesized to be on the pathway to an ozone effect and remain unexplained. The Panel also agreed with the investigators' conclusions that exposure to 70 and 120 ppb led to significant changes in lung function and two markers in the lung and blood that were consistent with ozone-induced injury to the airways. The pulmonary results in older adults are consistent with the results of other studies in younger volunteers showing lung effects after ozone exposures at concentrations resembling the current

Research Report 192, Part I

U.S. 8-hour ambient air quality standard for ozone of 70 ppb.

The Panel also agreed with the investigators that a major limitation of the study was that the participants were healthy. By design, participants were selected to have a normal body mass index and FEV₁; were able to perform moderate, intermittent exercise for three hours; and were able to abstain from a specified list of medications for one week. Thus, the study sample represented an older, but very healthy, mostly Caucasian segment of the population. Additionally, the study was limited — also by design — to acute exposures to primary ozone without reaction products and without co-exposure to other pollutants common in ambient air. Therefore, the observed lack of cardiovascular effects may not be generalizable to the overall adult population, which includes people who are less healthy and who are exposed to multiple pollutants. The emerging epidemiological evidence finding associations of cardiovascular effects with exposure to ozone may reflect susceptible members of the population who are unable to participate in clinical studies.

Because there was considerable variability in outcome values among participants, the Review Panel asked the investigators to evaluate whether a subgroup existed that showed larger changes in lung function or in sputum polymorphonuclear leukocytes after ozone

exposure than other subgroups and which may have also shown effects on the cardiovascular system that were not evident in the group as a whole. However, detailed statistical analyses did not find evidence of the presence of such a responder group.

CONCLUSIONS

The Multicenter Ozone Study in older Subjects was a large, well-conducted study in 87 healthy adults (55–70 years old) that showed the following important results: (1) there was no convincing evidence that a 3-hour exposure to 70 or 120 ppb ozone with moderate exercise affected cardiovascular endpoints in these healthy older adults; (2) short-term exposures at these low ozone concentrations did produce moderate pulmonary effects, showing results similar to previous studies in younger adults; and (3) no responder subgroup could be identified in which ozone elicited cardiovascular effects that were not evident in the group as a whole. The MOSES Review Panel agreed with the main findings of the study and commended the investigator teams for the high quality of the data and analyses. The respiratory effects observed after ozone exposure are consistent with the results of other studies showing such effects at current ambient ozone concentrations. Because the volunteers in this study were healthy, the results may not be generalizable to the overall adult population.

