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Cardiovascular function and ozone exposure: The Multicenter Ozone Study in oldEr Subjects (MOSES)

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ABSTRACT

Background: To date, there have been relatively few studies of acute cardiovascular responses to controlled ozone inhalation, although a number of observational studies have reported significant positive associations between both ambient ozone levels and acute cardiovascular events and long-term ozone exposure and cardiovascular mortality.
Objectives: We hypothesized that short-term controlled exposure to low levels of ozone in filtered air would induce autonomic imbalance, repolarization abnormalities, arrhythmia, and vascular dysfunction.
Methods: This randomized crossover study of 87 healthy volunteers 55–70 years of age was conducted at three sites using a common protocol, from June 2012 to April 2015. Subjects were exposed for 3 h in random order to 0 ppb (filtered air), 70 ppb ozone, and 120 ppb ozone, alternating 15 min of moderate exercise with 15 min of rest. A suite of cardiovascular endpoints was measured the day before, the day of, and up to 22 h after each exposure. Mixed effect linear and logit models evaluated the impact of exposure to ozone on pre-specified primary and secondary outcomes. Site and time were included in the models.
Results: We found no significant effects of ozone exposure on any of the primary or secondary measures of autonomic function, repolarization, ST segment change, arrhythmia, or vascular function (systolic blood pressure and flow-mediated dilation).
Conclusions: In this multicenter study of older healthy women and men, there was no convincing evidence for acute effects of 3-h, relatively low-level ozone exposures on cardiovascular function. However, we cannot exclude the possibility of effects with higher ozone concentrations, more prolonged exposure, or in subjects with underlying cardiovascular disease. Further, we cannot exclude the possibility that exposure to ambient ozone and other pollutants in the days before the experimental exposures obscured or blunted cardiovascular biomarker response to the controlled ozone exposures.

1. Introduction

Exposure to ozone in ambient air is a well-established cause of acute respiratory morbidity. About 80% of inhaled ozone reacts irreversibly with constituents of the respiratory tract lining fluid, with the remainder exhaled. Reactions with unsaturated lipids generate other reactive species on the airway surface that cause local oxidative stress, autonomic stimulation, acute inflammation, and epithelial cell injury

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(Bromberg, 2016). Although inhaled ozone itself does not penetrate beyond the respiratory tract surface, the locally generated ozonation products can provoke systemic effects.

Ozone is one of six pollutants for which the United States Environmental Protection Agency (U.S. EPA) sets a NAAQS to protect public health with an adequate margin of safety, as required by the Clean Air Act. The current ozone NAAQS, 70 ppb averaged over 8 h, was promulgated in October 2015 (U.S. EPA, 2015), and is based on an expanded body of clinical and epidemiological studies, which are reviewed in the U.S. EPA's Integrated Science Assessment (U.S. EPA, 2013). The main drivers of the standard were the respiratory effects observed in epidemiological studies and in environmental chamber exposure studies at concentrations as low as 70 ppb (Schelegle et al., 2009) and 60 ppb (Adams, 2006; Kim et al., 2011). In that 2013 Integrated Science Assessment of ozone, the U.S. EPA concluded that, in addition to respiratory morbidity, there is likely a causal relationship between short-term ozone exposure and total and cardiovascular mortality (U.S. Environmental Protection Agency, 2013). This was largely based on epidemiology studies (Ballester et al., 2006; Bell et al., 2004; Chan et al., 2006; Chang et al., 2005; Halonen et al., 2009; Lee et al., 2003; Link et al., 2013; Metzger et al., 2007; Mustafic et al., 2012; Rich et al., 2005; Rich et al., 2006a; Rich et al., 2006b; Rich et al., 2004; Rodopoulou et al., 2014; Shah et al., 2013; Shah et al., 2015; Szyszkowicz, 2008; Vedal et al., 2004), though not all studies agreed (Barnett et al., 2006; Corea et al., 2012; Franck et al., 2014; Fung et al., 2005; Goldberg et al., 2008; Sarnat et al., 2015; Symons et al., 2006; Tolbert et al., 2007; Zanobetti and Schwartz, 2006). Epidemiology studies of ozone may be confounded by other co-occurring pollutants such as particulate matter (PM). There have been a few studies of acute cardiovascular responses to controlled ozone exposure (e.g., 120 ppb for 2 h, 300 ppb for 3 h, some with intermittent exercise) in generally healthy subjects, with inconsistent findings (Arjomandi et al., 2015; Barath et al., 2013; Brook et al., 2002; Brook et al., 2009; Devlin et al., 2012; Gong Jr. et al., 1998). Studies of older adults, who might be more susceptible, have not been reported.

The Multicenter Ozone Study in oldEr Subjects (MOSES) was a multicenter, controlled human exposure study of acute cardiovascular and pulmonary effects following controlled ozone exposure in 87 healthy non-smoking women and men (ages 55 to 70 years). Subjects were exposed in random order to filtered air containing 0 ppb, 70 ppb, or 120 ppb ozone for 3 h while alternately exercising and resting for 15 min. We hypothesized that short-term exposure to ambient levels of ozone would induce autonomic imbalance (i.e., altered heart rate variability [HRV]), cardiac repolarization abnormalities, arrhythmia, and vascular dysfunction. Analyses examining these hypotheses are presented below. In a comprehensive and detailed report of MOSES available online, significant dose-related ozone effects on lung function, airway inflammation, and lung injury in these same subjects were observed (Frampton et al., 2017; Arjomandi et al., 2017).

2. Materials and methods

2.1. Study population

MOSES was conducted in three clinical centers: University of Rochester Medical Center (URMC; n = 32), University of North Carolina (UNC; n = 29), and the University of California, San Francisco (UCSF; n = 26). The New England Research Institute (NERI) served as the Data Coordinating and Analysis Center. The study was approved by institutional review boards at each center, and written informed consent was obtained from all subjects. Participants were healthy non-smokers, 55 to 70 years of age, with a normal 12-lead resting ECG and normal spirometry. Detailed inclusion and exclusion criteria are provided in Appendix A.

After recruitment using local postings and advertisements, participants attended a screening and a training visit to confirm they met inclusion and exclusion criteria, and to establish they were able to safely exercise at a level sufficient to achieve the target minute ventilation (V'E) of 15–17 L(BTPS)/min/m^2 BSA (body surface area). Subjects were not studied within 6 weeks of a respiratory infection.

2.2. Study design and protocol

Using a randomized, double-blind, crossover design, each subject participated in three exposure sessions, separated by at least 2 weeks, each involving a 3-h exposure to 120 ppb, 70 ppb, or 0 ppb ozone (filtered air). Selected baseline measurements were performed the day before exposure and the subject spent the night in a nearby, non-smoking hotel room. The next morning after a light breakfast, blood pressure (BP) and other vital signs were measured, Holter monitor leads were attached, and resting ECG was recorded. The Holter recording then continued for 24 h. Exposure (alternating 15 min of exercise and rest at the workload determined during the training visit) started between 8:00 and 8:45 am. V'E was measured intermittently during exercise with workload adjusted as needed to maintain V'E in the targeted range. Sitting BP was measured at rest 5 min before the third and fifth exercise periods.

Immediately to approximately 15 min after exposure, vital signs and HRV were measured and a low-fat lunch was provided. Approximately 3 h after the end of the exposure, ECG recordings were made, vital signs were measured, venous blood was obtained, and brachial artery ultrasound (BAU) was performed. The subject went home at 4:00–4:30 pm wearing the Holter monitor, and returned at ~8:00 am the next morning when vital signs and ECG recordings were made, and the Holter leads were removed.

2.3. Holter analysis

ECG monitoring utilized 12-lead Holter recorders (Mortara H12+ Holter recorder; Mortara Instruments, Inc., Milwaukee, WI). At the beginning of each recording and at designated intervals, subjects reclined in a dark, quiet room for 10 min to acquire data without the influences of activity or changes in body position. The last 5 min of each 10-min interval was marked for detailed analysis. All Holter cards were shipped to the Holter (ECG) Core Laboratory at URMC for annotation and analysis. ECG outcomes are provided below, with **primary outcomes bolded**. Unless otherwise specified, they are reported as 5-min averages (during supine resting periods) and 24-h averages (entire recording). See Appendix B for additional details.

2.3.1. Autonomic function

In the time domain, we measured the NN interval (interval in ms between successive normal beats), heart rate (HR in beats/min using the formula 60,000/NN), **RMSSD** (square root of the mean of the sum of squared differences between adjacent NN intervals), and SDNN (standard deviation of all NN intervals). In the frequency domain, we measured **HF** (high frequency power, 0.15–0.40 Hz), **LF** (low frequency power, 0.04–0.15 Hz), and LF/HF ratio.

2.3.2. Repolarization

We measured QT interval duration corrected for heart rate (QTc) using Bazett's formula and T wave magnitude (amplitude between the J wave [QRS end] and the end of the T wave). T-wave magnitude encompasses ST and T wave changes, better reflecting overall ST-T wave morphology in comparison to the traditional **T wave amplitude**. To compare our values to the more standard **T wave amplitude**, we converted our T wave magnitude values to T wave amplitude values using: **T wave amplitude** (uV) = 4 ∗ T wave magnitude (uV ∗ ms)/sqrt [8 ∗ JT (ms)](See Appendix B). We also measured **ST segment changes in ECG lead V5** and ST segment changes in leads II and V2.