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Pulmonary function responses to ozone in smokers with a limited smoking history

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ABSTRACT

In non-smokers, ozone (O_3) inhalation causes decreases in forced expiratory volume (FEV_1) and dead space (V_D) and increases the slope of the alveolar plateau (S_N). We previously described a population of smokers with a limited smoking history that had enhanced responsiveness to brief O_3 boluses and aimed to determine if responsiveness to continuous exposure was also enhanced. Thirty smokers (19 M, 11 F, 24 ± 4 years, 6 ± 4 total years smoking,4 ± 2 packs/week) and 30 non-smokers (17 M, 13 F, 25 ± 6 years) exercised for 1 h on a cycle ergometer while breathing 0.30 ppm O_3. Smokers and non-smokers were equally responsive in terms of FEV_1 (−9.5 ± 1.8% vs −8.7 ± 1.9%). Smokers alone were responsive in terms of V_D (−6.1 ± 1.2%) and S_N (9.1 ± 3.4%). There was no difference in total delivered dose. Dead space ventilation (V_D/V_T) was not initially different between the two groups, but increased in the non-smokers (16.4 ± 2.8%) during the exposure, suggesting that the inhaled dose may be distributed more peripherally in smokers. We also conclude that these cigarette smokers retain their airway responsiveness to O_3 and, uniquely, experience changes in V_D that lead to heterogeneity in airway morphometry and an increase in S_N.

Introduction

Ground-level ozone (O_3) is an urban outdoor pollutant generated by a photochemical reaction between atmospheric oxygen with nitrogen oxides and hydrocarbons from automobile and industrial emissions (Kasibhatla and Chameides, 2000). Exposure to ground-level O_3 causes impairments in airway function in healthy non-smokers. Classically, continuous exposures to O_3 with constant or intermittent exercise have been used to predict how populations might respond to environmental exposure (Foster et al., 1987, 2000; Reeser et al., 2005), and the forced expired volume in 1 s (FEV_1) is frequently used to evaluate changes in airway function. For example, non-smokers exposed to 0.10–0.40 ppm O_3 with intermittent, moderate exercise experience dose-dependent decreases in FEV_1 on the order of 0.2–0.8 L (McDonnell et al., 1983).

This decline in FEV_1, is caused by both cholinergically-mediated bronchoconstriction and impairment in inspiratory capacity (Hazucha et al., 1989). While FEV_1 is an important index of global lung function, it cannot discriminate between bronchoconstriction that occurs uniformly throughout the lung and focal bronchoconstriction. Non-uniform or heterogeneous bronchoconstriction results in non-uniform alveolar emptying, which can be evaluated using gas washout methods (Schulzke and Frey, 2013; Wongviriyawong et al., 2013). To that end, we previously evaluated the normalized slope of the alveolar plateau of the capnogram (S_N) to determine whether O_3 exposure increases ventilation heterogeneity. Non-uniform bronchoconstriction results in non-uniform alveolar emptying, translating to an increase in S_N. We previously found S_N to be increased by continuous ozone exposure in non-smokers (Taylor et al., 2006).

Longitudinal O_3 bolus uptake measurements have also been a valuable tool for understanding how O_3 is distributed in the respiratory system. During quiet breathing, the majority of O_3 is removed by the epithelial lining of the nose and pharynx (Kabel et al., 1994). At flow rates similar to those that occur during moderate exercise, ~80% of O_3

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is removed in the conducting airways (Bush et al., 1996a; Hu et al., 1992, 1994). This technique also has been used to investigate the effect of sex, ventilation rate, and previous exposure to O_3, SO_2 and NO_2 on the longitudinal distribution of O_3 in healthy non-smokers (Asplund et al., 1996; Bush et al., 2001; Hu et al., 1992, 1994; Rigas et al., 1997).

We previously investigated whether the distribution of inhaled O_3 is different in young cigarette smokers with a limited smoking history (Bates et al., 2009) and found it to be similar to comparable non-smokers. Unexpectedly, we also found that these smokers experienced a small decline in FEV_1 and increase in S_N in response to these brief O_3 boluses (Tsang et al., 2000). Although these changes were subtle, the total dose of delivered O_3 was also small. We speculated that this population of smokers would have a more pronounced response to continuous O_3 exposure, when the total dose of O_3 is greater.

To test this hypothesis, we studied the 30 college-aged smokers and 30 non-smoking controls previously evaluated using our O_3 bolus technique (Bates et al., 2009). Pulmonary function was assessed using forced spirometry and exhaled capnography after 60 min of steady state exercise with either room air or 0.30 ppm O_3. As before, we used forced spirometry to evaluate FEV_1 and the capnogram to evaluate S_N. To further characterize changes in pulmonary function, we also measured forced vital capacity (FVC) and the ratio of the anatomic dead space volume, (V_D) to the tidal volume (V_D/V_T). The major findings of this work are that young smokers and non-smokers experience similar decrements in FEV_1 and FVC, but smokers experience greater decrements in S_N and V_D. These greater decreases in V_D translate to a larger decrease in V_D/V_T in smokers than non-smokers, leading to an increased delivery of O_3 to the respiratory airspaces.

Methods

Subject selection and health screening. Sixty human research participants (30 smokers, 30 non-smokers) previously evaluated using our O_3 bolus technique were enrolled in this study. After reviewing and signing an informed consent, each participant received a physical exam and completed medical and smoking history questionnaires. Forced spirometry was performed according to previously published standards (Miller et al., 2005) in order to assess FEV_1 and forced vital capacity (FVC). As before, smokers were admitted into the study if they reported daily cigarette use and a smoking history ≥ 1 pack–year but ≤ 15 pack–years. Levels of cotinine, a metabolite of nicotine with a half-life of 20 h, were also determined from the blood samples in order to validate reported smoking history. From an assay of their plasma cotinine drawn on two visits (Bates et al., 2009), subjects with concentrations greater or equal to 32 ng/mL on both occasions were judged to be smokers (Benowitz, 1996), This objective classification was consistent with the participants' self-reported use of tobacco. Only subjects who reported no history of smoking and were negative for plasma cotinine were classified as non-smokers.

To evaluate each participant's ability to exercise safely and exclude participants with likely cardiovascular disease, blood was drawn and analyzed for cholesterol and triglycerides, and a maximal exercise tolerance test with a 12-lead ECG was performed. Additional inclusion criteria included an $FEV_1/FVC > 0.70$ and $FEV_1 > 80\%$ using Knudson predicted values (Knudson et al., 1976), no history or presentation of cardiovascular or respiratory disease, and no regular use of either prescription medication (except hormonal birth control) or over-the-counter pain relievers in the 48 h prior to exposure. Female participants were given a urine hCG test prior to each session to exclude pregnancy. All aspects of the study, including medical oversight, measurements and administration of informed consent, were conducted at the General Clinical Research Center and approved by the Office of Research Protections of the Pennsylvania State University.

Exposure apparatus and exercise protocol. After screening, the volunteers participated in two sessions in which they were exposed to either room air or 0.30 ppm O_3. Control and O_3 exposures were performed on different days and in random order, with at least one week between exposures, to limit carry-over and sequence effects. Although smokers were not asked to abstain from tobacco use before the session, they did not smoke during the minimum of 45 min that elapsed from the time they reported to the laboratory and the beginning of the exposure. We did not ask if they had smoked before reporting to the laboratory. During the O_3 exposure session, the participant exercised for 1 h on a cycle ergometer. Workload was adjusted to elicit a target minute ventilation (V_E) of 15 L/min/m^2 of body surface area (Verbraeken et al., 2005). Throughout the session, ventilation and O_3 concentration were detected immediately proximal to the subject's breathing mask using previously described instrumentation (MacDougal et al., 1998; Reeser et al., 2005).

At the beginning of the exposure, the participant sat on a cycle ergometer, donned an oral breathing mask, and pedaled at a rate of 60 revolutions/min at a workload of 25 W. Thereafter, workload was gradually increased to 10–25 W/min until the target V_E was reached. On average, this warm-up period required 3 min. Once the target V_E was reached, the oral breathing mask was attached to the O_3 generation system and the one-hour exposure began. The exposure period was followed by a three minute cool down period during which the oral breathing mask was removed and the participant cycled at 25 W while breathing room air.

The ventilation and O_3 signals were recorded throughout the exposure using a digital data acquisition system. After completion of the session, the respired flow signal was analyzed by an automated computer program (LabVIEW, National Instruments Corporation, Austin, TX) to determine the inhaled and exhaled tidal volumes and the period of each breath. Data for each individual breath were then combined and averaged over five-minute periods to determine average inhaled tidal volume (V_T), breathing frequency (f), and V_E. The product of the respired flow and inspired and expired O_3 concentration signals were integrated to compute O_3 uptake for each individual breath. These data were also combined over five-minute data acquisition intervals to determine the average fractional uptake efficiency (UE), defined as the ratio of retained dose to inhaled dose.

Ozone was generated using a commercially available generator (03V1-0, OREC, Phoenix, AZ) that operates by passing room air over an ultraviolet lamp (McDonnell et al., 1983; Reeser et al., 2005; Taylor et al., 2006). Ozonated air was produced at a rate of 250 L/min and delivered to the inspiratory port of the non-rebreather valve via sidestream sampling of the ozonated air stream. Ozonated air generated in excess of the participant's demand was vented to the outside environment. During the room air exposure session, the flow path of room air through the O_3 generator were identical to that used during the O_3 exposure, except that the ultraviolet light within the generator was turned off. Thus, the only difference between the two sessions was that the inspired air stream was not ozonated during the control session.

Measurements of lung responses. Spirometry and capnography were performed immediately before and after exercise. A clinical-grade spirometer (KoKo model, Ferraris) was used to measure pre- and post-exposure values of FEV_1 and FVC according to standardized techniques (Miller et al., 2005). A minimum of three maneuvers were performed and repeated until the two largest values of FEV_1 and FVC were within 0.15 L.

Single-breath capnograms were measured to determine V_D and S_N using a previously described method and equipment (Bates et al., 2009; Taylor et al., 2005). Briefly, after exhaling to functional residual capacity, participants completed two sequential breaths while controlling their respiratory air flow at 250 mL/s. The first breath consisted of 750 mL inhaled and exhaled volumes. The second breath consisted of